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Award Number:
W81XWH-08-2-0131

TITLE:
MISSION CONNECT MILD TBI TRANSLATIONAL RESEARCH CONSORTIUM

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REPORT DATE:
August 31, 2010

TYPE OF REPORT:
Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

X Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
8-31-2010	Annual	08-01-2009-07-31-2010

4. TITLE AND SUBTITLE

MISSION CONNECT MILD TBI TRANSLATIONAL RESEARCH CONSORTIUM

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-08-2-0131

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

John Holcomb, MD

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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Houston, TX 77030-3900

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

US Army Med Research Acq Act
820 Chandler Street
Fort Detrick, MD 21702-5014

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This report outlines the overall activities of the Mission Connect Mild Traumatic Brain Injury (TBI) Translational Research Consortium during the time period of August 1, 2009 through July 31, 2010. Dr. John Holcomb serves as the Initiating Investigator and Co-Director of the Consortium, along with Dr. Claudia Robertson, Co-Director.

This report also provides a summary of consortium activities to date, reviews issues related to the leadership of the consortium, and describes Dr. Holcomb's scientific participation as a co-investigator on both laboratory and clinical projects. In addition, a detailed summary of each consortium project is provided on pp. 17 - 36.

In our second year of working together as a Consortium, we have made significant progress. Work is proceeding on all projects, with progress on all projects. Our Working Group structure is providing an effective mechanism for scientific collaboration and coordination of related projects. We have successfully initiated patient enrollment in our clinical protocol and by adapting to the unique issues at each institution have doubled enrollment over the last 3 months.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE		39	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION: This report outlines the overall activities of the Mission Connect Mild Traumatic Brain Injury (TBI) Translational Research Consortium during the time period of August 1, 2009 through July 31, 2010. Dr. John Holcomb serves as the Initiating Investigator and Co-Director of the Consortium, along with Dr. Claudia Robertson, Co-Director.

BODY: This report will provide a summary of consortium activities to date, review issues related to the leadership of the consortium, and describe Dr. Holcomb's scientific participation as a co-investigator on both laboratory and clinical projects. In addition, a detailed summary of each consortium project is provided on pp. 17 - 36.

Consortium Summary: In the first year of working together as a Consortium, we were primarily occupied with start-up activities, for both individual projects and for the organization of the Consortium. In Year 2, the focus of this report, the Consortium membership has worked together to accomplish the following:

- We have solidified the organizational structure of the Consortium, including regular meetings of the Working Groups (see Consortium Leadership and Working Groups discussions below) and quarterly general meetings of all Principal Investigators.
- A transition of the Initiating Investigator role has been accomplished from Dr. Alex Valadka, who departed in August of 2009, to Dr. John Holcomb, who assumed this role in October, 2009.
- A review procedure by the CDMRP-appointed External Review Board (EAB) has been implemented, including:
    - a meeting of EAB members and Consortium Investigators in Chicago, held on October 16, 2009
    - quarterly conference calls of the EAB members that provide feedback to Consortium Investigators
- Consortium Investigators have made formal written responses to all requests for additional information and clarification of projects by EAB members and have incorporated EAB feedback into projects.
- We have established contacts and collaborative relationships with a number of military clinicians to provide guidance and input on the clinical projects. See pp. 15-16 for further discussion of this.

Consortium Leadership: As Initiating Investigator, Dr. Holcomb holds the position of the chair of the Executive Committee and of the Steering Committee and works with the Administrative Core and the Research Coordinator, Dr. Emmy Miller. The organizational structure (see Figure 1, p. 5) of the Consortium has been a key factor in our ability to manage the project, to promote collaboration and teamwork among investigators, and to facilitate on-going Consortium activities.

The Executive Committee (Drs. Holcomb – UT-H, Robertson – BCM and Dash – UT-H) are joined by Drs. Levin – BCM, Perez-Polo – UTMB, and Hulsebosch – UTMB to make up the Steering Committee of the Consortium. Regular monthly meetings of the Steering Committee are occurring, with valuable discussion and problem solving. In addition to general coordination and oversight, the Steering Committee has focused on 3 major issues in Year 2:

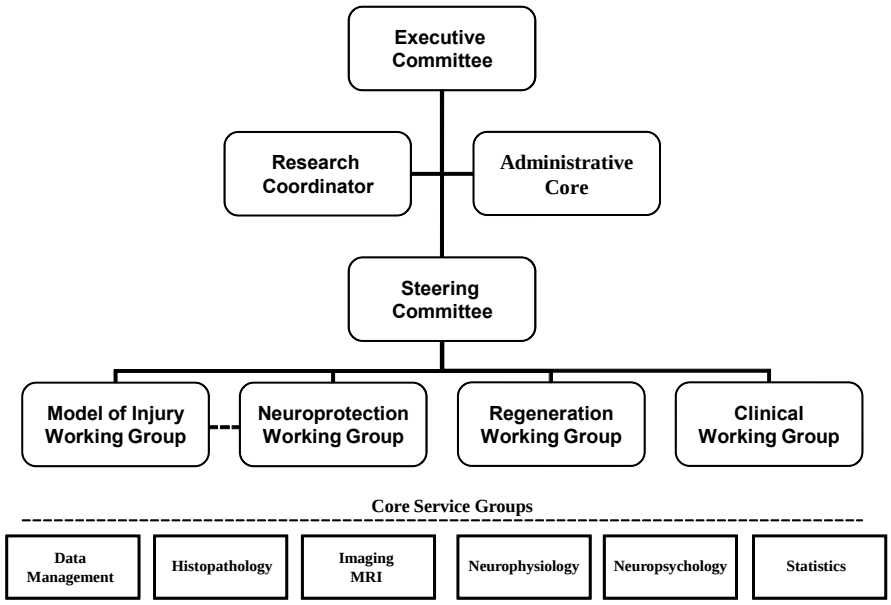
1. Transition the Initiating Investigator role from Dr. Alex Valadka to Dr. John Holcomb,
2. Responding to feedback from the EAB
3. Ensuring effective collaboration among Consortium Investigators through the Working Group structure and quarterly meetings of the Investigators.

There have been 5 meetings of the full Investigator group during Year 2. These meetings addressed:

- 9/21/2009 – Preparation for the EAB meeting in Chicago

- 11/4/2009 – Reviewing and preparing responses to EAB feedback from the Chicago meeting
- 1/29/2010 – Continued response to EAB feedback, discussion of projects and related issues prior to February quarterly report
- 4/22/2010 – Discussion of projects and related issues prior to the May quarterly report
- 6/23/2010 – Discussion of projects and related issues; preparation for Annual Reporting process

Figure 1
The Mission Connect Mild TBI Translational Research Consortium



Working Groups: The Working Groups are the primary organizational unit of the Consortium, and group meetings are taking place, although the frequency varies by the needs of the groups.

Model of Injury Working Group and Neuroprotection Working Group
Chair: Dr. Doug DeWitt – Model of Injury
Chair: Dr. Regino Perez-Polo
(Summary provided by Dr. DeWitt)

Since the Model of Injury Working Group and the Neuroprotection Working have a significant overlap in membership and goals, these groups have been meeting jointly. The Model/Neuroprotection Working Group (M/NWG) met on 20 January, 2010, 23 March, 2010 and then, informally, after the Mission Connect mTBI Consortium Investigators meeting on 22 April, 2010. At the 20 January, 2010 meeting, it was decided that the Model and Neuroprotection Working Groups would meet jointly since many of the members of the MWG also were members of the Neuroprotection Working Group. Prior to each meeting, Drs. DeWitt and Perez-Polo, the Chairs of the Model Working Group and Neuroprotection Working Group, respectively, will work together to compile an agenda that meets the needs of both groups. This report is a compilation of the results of the meetings of the combined committees.

M/NWG Goals:
1. To ensure that a standard protocol is developed and used consistently for each of the experimental models used by all Mission Connect Translational MTBI Translational Research Consortium investigators.

2. To thoroughly characterize the models by establishing and performing a standard group of physiological, histopathological and behavioral outcome measures on all four models.

3. To evaluate the efficacy of the neuroprotective agents being tested during the current period of support of the Mission Connect Translational MTBI Translational Research Consortium and to identify potentially neuroprotective agents or strategies for future testing.

The table below is a summary of the experimental traumatic brain injury (TBI) models that are used by the investigators of the Mission Connect Translational MTBI Translational Research Consortium. The 1^{o} Investigator is investigator who developed the experimental model and/or is providing injured animals for the Investigators who are studying the effects of that type of TBI.

Mission Connect mTBI Consortium experimental TBI models		
Model	**1^{o} Investigator**	**Investigators**
Fluid percussion injury	DeWitt	Dash, DeWitt, Perez-Polo, Grill
Controlled cortical impact	Robertson	Robertson
Rasband blast injury	Rasband	Rasband, Grill, Dash
Vandenberg blast injury	DeWitt	DeWitt, Grill, Dash, Perez-Polo

mTBI Model Standardization

Based on discussions during the meetings, the MWG recommended that the following data will be acquired for all of the experimental TBI models:

1. Mean arterial blood pressure & heart rate
2. Cerebral perfusion (e.g. laser Doppler flowmetry, ^{14}C-iodoamphetamine autoradiography
3. Vestibulomotor outcome (e.g. beam balance/beam walking, RotaRod performance)
4. Latency to return of the righting reflex (a measure of duration of unconsciousness after injury)
5. Spatial or working memory function (e.g Morris water maze or Barnes maze performance)
6. Anxiety (e.g. startle reflex, open-field behavior)
7. Histopathological outcome (e.g. counts of surviving neurons in cortex and hippocampus)
8. Blood brain barrier permeability (albumin and/or dextrans of different sizes)
9. APP, BDNF, GFAP immunohistochemistry.

These data will be used to characterize, compare and contrast the pathophysiological effects of the experimental TBI models. In addition, each investigator may make additional measurements relevant to his/her specific project.

The table of assignments for the model characterization studies was completed (below).

Measurement	**Model**	**Investigators (animals/measurements)**
MAP, HR	FPI	DeWitt/DeWitt
	CCI	Robertson/Robertson
	EBI	DeWitt/DeWitt
	PBI	Rasband/Robertson
Cerebral perfusion	FPI	DeWitt/DeWitt
	CCI	Robertson/Robertson
	EBI	DeWitt/DeWitt
	PBI	Rasband/Robertson

Vestibulomotor	FPI	DeWitt/DeWitt
	CCI	Robertson/Robertson
	EBI	DeWitt/DeWitt
	PBI	Rasband/Rasband
Righting reflexes	FPI	DeWitt/DeWitt
	CCI	Robertson/Robertson
	EBI	DeWitt/DeWitt
	PBI	NP
Memory	FPI	DeWitt/DeWitt
	CCI	Robertson/Robertson
	EBI	DeWitt/DeWitt
	PBI	Rasband/Rasband
Anxiety	FPI	DeWitt/DeWitt
	CCI	Robertson/Robertson
	EBI	DeWitt/DeWitt
	PBI	Rasband/Rasband
Surviving neurons	FPI	DeWitt/DeWitt
	CCI	Robertson/Robertson
	EBI	DeWitt/DeWitt
	PBI	Rasband/Rasband
Blood-brain barrier	FPI	DeWitt/Grill
	CCI	Robertson/Grill
	EBI	DeWitt/Grill
	PBI	Rasband/Grill
GFAP, APP, BDNF IHC	FPI	DeWitt/Grill
	CCI	Robertson/Grill
	EBI	DeWitt/Grill
	PBI	Rasband/Grill
APP – amyloid precursor protein; BDNF – brain derived neurotrophic factor; CCI – controlled cortical impact; EBI – explosive blast injury; FPI – fluid percussion injury; GFAP – glial fibrillary acidic protein; IHC – immunohistochemical; NP – not practical; PBI – pressure blast injury		

Blast-induced Brain Injury Model Characterization:

David Ritzel, a widely recognized expert on the physics of blast injury, has agreed serve as a consultant for the studies of the effects of blast injury on the brain using the Vanderberg blast device. The Vandenberg device was designed by Dr. DeWitt in consultation with Mr. Edward Vandenberg, an expert gunsmith. The device was constructed by Mr. Vandenberg's company, VandenBerg Customs.

Mr. Ritzel traveled to Galveston 23 – 25 June, 2010 to present a day-long workshop on the physics of blast injury. Several mTBI Consortium members attended the workshop. During his visit, Mr. Ritzel reviewed high-speed digital videos of the Vandenberg device and suggested modifications that will be performed by he and Mr. Steve Parks of ORA, Inc., Fredericksburg, MD. In addition, Mr. Ritzel and Mr. Parks will construct a unique table-top conical blast generator. The device, which can be driven by either compressed helium or an oxyacetylene explosive gas mixture, will be constructed so that only the rat's head is exposed to the effects of blast pressures. When the modifications of the Vandenberg device and the

conical blast generators are complete, we will have the only laboratory with the capability to compare and contrast the effects of explosive blast injury produced by the detonation of gunpowder or oxyacetylene with those of blast pressures generated by compressed gas.

Data Type, Collection & Storage:

The M/NWG discussed and made recommendations related to the following questions:

1. What constitutes "mTBI" for each experimental model? There was a discussion of the need to identify the primary endpoints that will be used to determine levels of mTBI for each model. A distinction between surrogate (e.g. righting reflexes) and primary (e.g. Fluoro-Jade (FJ) staining) was defined. For the FPI, CCI, and EBI (Vandenberg) models, righting reflex (RR) will be used as a surrogate endpoint for defining mTBI. Dr. Robertson noted that level of consciousness was one of the main determinants of injury level in humans. Since RR is a measure of level of consciousness, it is useful as both a surrogate and primary endpoint for defining mTBI. In addition, investigators may use other primary endpoints that are relevant to their studies to further define mTBI. For example, RR correlates well with numbers of FJ-stained hippocampal neurons, a primary endpoint for defining mild FPI. In contrast, the correlation between RR and FJ staining is not yet known for EBI. For the immunohistochemical measurements made in Dr. Grill's laboratory, mTBI may be defined based on degree of BBB compromise (e.g. albumen staining) or APP expression. The correlation between these endpoints and RR is not yet known. In addition, RR suppression measurements aren't feasible for the PBI model used by Dr. Rasband due to the depth of anesthesia required. Therefore, Dr. Rasband will define mTBI based on BBB compromise or some other histochemical measurement.

2. Where will the raw data be stored? The M/NWG members recommended that the raw data remain with the investigators who collected it. Dr. Miller advised that the Consortium website was not structured for the storage of large quantities of raw laboratory data.

3. Where are descriptive stats (e.g. mean ± sem, n) stored? The M/NWG members recommended that compiled descriptive statistics (e.g. means, standard errors/deviations) for numerical data (e.g. MAP, ICP, CBF) for each model be stored on the Consortium website. Dr. Miller confirmed that the website would be suitable for storage of mean data. The M/NWG recommended that the primary investigators (see table above) will collect, compile and upload descriptive statistics for their models.

4. What data are shared and where? As stated above, descriptive statistics for numerical data will be available on the Consortium website, although the design and plan for this still needs to be developed.

5. Where will images be stored (e.g. IHC, MRI)? The large size of the image files precludes storage on the Consortium website, images will remain in the laboratories where they were collected.

6. Behavioral data: Numerical data (e.g. Morris water maze latencies, swim speeds, time in quadrant) from the behavioral assessments would be treated the same as other numerical data.

7. Non-standard Data Collection and/or Sharing: "Non-standard" data are defined as those collected by each investigator as part of his/her individual project. Since each project has specific aims related to the specific hypotheses of that project, data not related to model characterization will be collected by each investigator. These data will remain in the laboratory in which they were collected. However, should an investigator find that the results of their experiments suggest an additional variable that might prove valuable for model characterization, he/she may recommend that the variable be added to the standard data set. For example, should a particular biomarker prove especially sensitive/specific some aspect of mTBI, the investigator may suggest that the biomarker be added to the standard model characterization data set.

The M/NWG would then decide whether to recommend that the biomarker be added to the standard model characterization data set.

Additional Behavioral Assessments

Among the most common and persistent cognitive problems reported by patients with mTBI are deficits in executive functioning and attention. These are difficult functions to evaluate in rodents. In order to develop more effective assessments of executive functioning and attention, Dr. DeWitt been in contact with Robert Hamm, Ph.D. of the Virginia Commonwealth University Medical Center and Tim Schallert, Ph.D. of the University of Texas at Austin. Drs. Hamm and Schallert are internationally respected authorities on behavioral assessments in rodents. Both investigators agreed to consult on measures of higher level cognitive functioning in rodents. In addition, Dr. Hulsebosch has invited Mark Whiting, Ph.D. of Radford University to travel to Galveston to present a workshop and seminar on behavioral assessments in rodents on 5 and 6 August, 2010. Dr. Whiting gave an excellent presentation on novel behavioral measures at the recent National Neurotrauma Symposium in Las Vegas, NV.

Summary

1. The Model and Neuroprotection Working Groups (M/NWG) have decided to meet jointly to pursue mutual goals and to minimize the workload for investigators that are actively involved in both groups.

2. The M/NWG identified a set of physiological, histopathological and behavior variables that will be measured in the four models of mTBI used by the mTBI Consortium investigators.

3. The M/NWG recommended that raw data and images remain in the laboratories in which they were collected. Mean numerical data for the model validation variables (item 2) will be compiled by the investigators who collect the data and made available to all mTBI Consortium investigators via the Consortium website.

4. Dr. DeWitt has been working with Mr. David Ritzel and Dr. Steven Parks to more accurately evaluate and modify the blast wave characteristics of the Vandenberg device.

5. Drs. DeWitt and Hulsebosch have been working with nationally and internationally renowned experts on behavioral assessments in rodents to evaluate and, if necessary, improve the behavioral assessment tasks used by Consortium investigators.

Restore Function Working Group
Chair: Dr. Michael Friedlander
(Summary of provided by Dr. Friedlander)

The Restore Function group has held two formal meetings in the last year, but many of the Investigators are working in the same lab or in near-by labs, as well as interacting frequently via phone and email to discuss the individual and collective successes and remaining challenges that they each face, and how they are addressing them. The collective application of standardized mTBI protocols and the injury models group deliberations and actions have been considered as were the strengths and weaknesses of the individual vs. core based approaches to analysis of the anatomical extent of contusions. The group has made suggestions to facilitate the progress of the rodent injury protocol evaluation, the processing of tissue, the integration of the behavioral and physiological analysis of NHPs and the strategies for facilitating the

enhanced capture and delivery of subjects from the study nurses for the human protocols. A brief overview of each Investigator in this group is provided below.

Dr. M. Rasband: The Statement of Work (SOW) has been revised to request consideration of utilization of mice in experimental protocols; received recent notification to utilize rats only in protocols an is complying with notification; has re-submitted another revised SOW with utilization of rats only; he is continuing to develop blast model and applying multiple immuno-staining protocols to evaluate extent and nature of mTBI and sharing with other members of the restore function team.

Dr. S. LaConte: Has had challenges with recruitment of participating human subjects; has received several calls from study nurses to follow up with potential subjects (3 control subjects and one mTBI patient); upon consideration of the study, the mTBI subject decided not to participate; One control subject was run on full protocols and has returned for longitudinal follow up study; data for control subject are being analyzed; there has been progress in learning how often patients arrive at participating hospital for CT scans that have potential mTBI – follow up discovery with study nurses to increase subject pool have been planned and implemented.

Dr. A. Tolias: Had encountered problem with infection in non-human primates (NHPs) participating in study that are being trained – infection has been contained; one NHP has been fully trained on a learning task; a second NHP has been prepared for implantation with stimulating electrodes.

Dr. S. Smirnakis: 2 NHPs have been trained for the project; a graduate student has joined the project; interface system has been developed and implemented to run programs; fMRI protocols for NHPs have been optimized; NHPs are undergoing training on fixation tasks that are necessary for running the behavioral protocols – animals have progressed well on fixations task learning.

K. Tolias: collaboration with Dr. C. Robertson on studies using impact injuries in mice; evaluation of contusion size with immunostaining (GFAP, RhoA, BCR, ABP) while evaluating expression of plasticity favoring and restricting knockout animals; has carriedout developmental studies of wild type and various knockout mice; animals are being evaluated for postsynaptic dendritic spine maturity and density; have observed higher density and larger dendritic spines in knockouts; preliminary findings that Rho knockout animals have apparently smaller contusion extent and reduced GFAP staining; some animals are being evaluated histologically by core facility (Dr. Grill) but resources are limited so animals also being evaluated in Dr. Tolias' lab for histological analysis of contusion extent; in BCR KO (global knockout animals including in neurons and in glia) mice that would be expected to have enhanced capacity for synaptic plasticity and potential recovery after injury, preliminary findings are suggesting that the recovery is worse – astrocytes seem to be hyper-activated based on more disorganized cytoskeletal elements and response on scratch assay.

Dr. M. Friedlander: development and refinement of protocols for the induction of the potentiating form of long term synaptic plasticity (LTP), particularly with higher frequency synaptic stimulation. Key of experimental goals is to maintain constant stimulation epoch duration (15 minutes) and constant number of synaptic stimulation pulses (1,500) during this period while varying only either stimulation frequency (e.g. 1 Hz, 10Hx, etc) or stimulation temporal pattern (e.g. perfectly regular stimulation pattern with all equal interstimulus intervals, very irregular Poisson distributed stimulation pattern with highly irregular inter-stimulus intervals or variably regular stimulus pattern with increasing variance of interstimulus interval distribution about some mean interval). However, this approach had raised some challenges, particularly with the higher stimulation frequencies. For example, with 10 Hz synaptic stimulation with a perfectly regular interstimulus interval distribution (all 100 msec) when 1,500 pulses are applied continuously, the

stimulation epoch ends within in 2.5 minutes and the constant train of high frequency fatigues synaptic transmission such that the onset of true long term synaptic plasticity cannot be observed due to likely depletion of synaptic vesicles presynaptically. Thus, the Friedlander team has developed an approach that allows for sufficient recovery periods during the 10 Hz stimulation epoch to avoid synaptic fatigue, but keeps the overall epoch of synaptic stimulation constant and still have period of 10 Hz stimulation with the bulk of the interstimulus intervals identical at 100 msec. They have developed a variety of protocols to accomplish this and over the last reporting period, have identified a successful protocol. They are now using a discontinuous stimulation epoch that applies 100 pulses at 10 Hz (100 msec interstimulus intervals) for 10 seconds (100 stimuli) followed by a 53 second rest period that includes 4 probe test stimuli delivered at 0.1 Hz. Thus, the interval distribution is predominantly 100 msec (1,485 perfectly regular 100 msec intervals) with a small number (of 10 sec probe intervals (39). When they apply this approach with the regular 10 Hz stimulation protocol, they observe the following: during each of the 15 high frequency (10 Hz) stimulation epochs, the peak synaptic response decreases sequentially likely due to synaptic fatigue). However, during the probe trials, the synaptic responses grow gradually and over the course of the entire synaptic stimulation epoch, the synaptic response grows to a saturating strong LTP (~80%). Thus, they can now fit the responses with a series of rate constants describing multiple interacting kinetic processes including accumulating synaptic fatigue during individual stimulation epochs but we also can capture the development of the long term synaptic potentiation process. This approach is now serving as the basis of the application of appropriately comparative protocols to utilize regular and the increasingly irregular stimulation patterns to evaluate their effects on the full range of synaptic plasticity (from strong LTD to no change to strong LTP) in the control animals as well as in the mTBI animals.

Dr. Wu had initially encountered some difficulties with animal survival but has worked that problem out. She has evidence for significant neuroprotective effects through her cell injection paradigm.

Clinical Working Group/The Integrated Clinical Protocol
Chairs: Dr. Harvey Levin and Dr. Emmy Miller

(summary provided by Dr. Miller)

Overview: The Clinical Working Group meets the most frequently of the Working Groups, with 18 meetings held in Year 2. The primary focus of this group is the on-going management and monitoring of the Integrated Clinical Protocol (ICP), which consists of three observational studies (Specific Aims 2.1 – Levin PI, 2.2 – Papanicolaou PI, and 2.3 – Masel PI) and one Phase II randomized clinical trial (Specific Aim 3.1.2-7 Robertson -- PI) that will use a shared group of 200 MTBI subjects and 100 Orthopedic Injury (OI) control subjects. Subject enrollment was begun on February 4, 2010, and as of July 31, there are 15 active subjects, with 305 screened and excluded. A full discussion of the screening/recruitment activities and enrollment statistics is presented in Dr. Harvey Levin's report.

Challenges to Subject Screening and Enrollment: In the first part of Year 2, the Clinical Working Group tackled several logistical/operational issues that delayed the start of subject enrollment, including:

1. Obtaining the required approvals from the IRBs responsible for our two recruitment sites (Baylor College of Medicine for the Ben Taub General Hospital site; UT-Houston for the Memorial Hermann Hospital site) and from HRPO. All of the required approvals were in place by December 2009.
2. Due to the change in the Initiating Investigator, with Dr. Alex Valadka's departure in August and Dr. John Holcomb's assumption of this role in October, there were subsequent organizational issues and paperwork (i.e. revisions to reflect personnel changes and subsequent approvals of those) that resulted in a delay of several months in the initiation of subject enrollment.

3. At the same time as the change in PI status, we recognized that prior to the start of subject enrollment we had to resolve the issue of alcohol use by potential study subjects. Studies report that a substantial percentage of patients presenting in the Emergency Center (EC) with mild TBI have been drinking, with reports ranging from 10-50%.[1-4] This issue was of concern for two reasons.
    a. One was that we wanted our study population to correspond, as much as possible, to the military population sustaining mild TBIs while on duty, and thus largely not exposed to alcohol.
    b. A second concern was the impact that alcohol consumption could have on the cognitive testing portion of the protocol.

There were two exclusion criteria in the protocol specific to screening for the presence of alcohol: 1) Blood alcohol level (BAL) is available and exceeds 80 gm/dL (conforms to Texas legal limit of 0.08%), and 2) Documentation of clinical intoxication in the patient record. Despite these, there were still concerns that subjects might be enrolled that were under the influence of alcohol. After much discussion, the Clinical Working Group decided that only subjects with a documented BAL would be enrolled. This would be a temporary measure until we could add a portable breathalyzer test to our protocol. The results from these instruments have shown strong correlation with blood alcohol analyses ($r = 0.940$, $p < 0.001$).[5] Under this protocol amendment, approved in May 2010, we conduct a breathalyzer test after enrollment to determine if the subject had alcohol in their system and if so, how much. Thus, the results of this breathalyzer test do not serve as an exclusion criterion, since we already have two alcohol-related exclusions, but rather serve to guide study staff to delay cognitive testing by altering the sequence of study activities (i.e. do MRI first, then do testing), thus minimizing any effect of alcohol consumption on test results.

Once we began subject enrollment on February 4, 2010, we encountered a number of operational and logistical issues that resulted in very slow accrual of subjects. The Clinical Working Group was the forum for strategizing and problem-solving to resolve these issues, including:

Problem: The original plan for subject enrollment was a case-finding approach where Research Nurses were stationed during "peak hours" in the Emergency Centers (ECs) of the two enrollment sites, Ben Taub General Hospital (BTGH) and Memorial Hermann Hospital (MHH). These peak hour periods were identified from the sites' in patient Trauma Registry data.
Solution: We recognized the limitations of the approach and began running a full 24/7 on-call schedule in time for Memorial Day week-end (May 28-31) and have continued to do so.
Solution: In addition, we have shifted from a case-finding method of identifying subjects to a combination case-finding/referral system. We have prepared and posted informational flyers in the ECs and provided pocket-cards (See Appendix 1) to encourage EC personnel to notify the on-call Research Nurse about potential subjects. The Research Nurses continue with their aggressive case-finding, but we are also getting referrals and anticipate that this will increase over time. In addition, our Research Nurses have remote access for the EC patient registration systems at both enrollment sites, so they can do some basic screening using this tool.

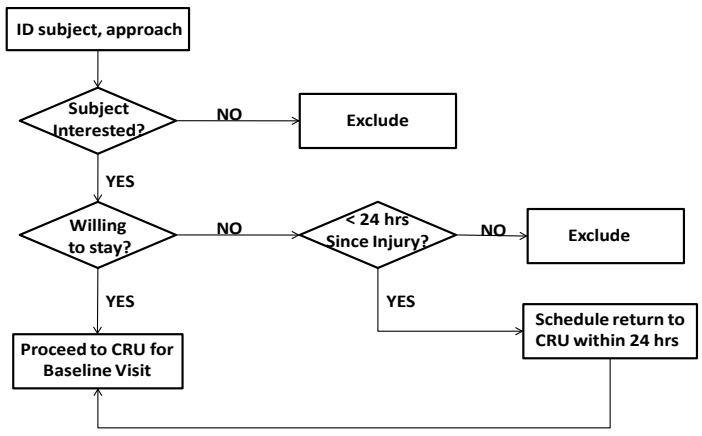
Problem: In the Integrated Clinical Protocol, once subjects have been discharged from the Emergency Center, they are transported to the Memorial Hermann Clinical Research Unit (CRU) for all Baseline research procedures except the MRI, which is done at the UT MRI Center. The CRU is only open during business hours on week-days, but we were initially able to negotiate limited after-hours and Saturday access, with the requirement that a CRU nurse had to be present (i.e. work overtime). It quickly became clear that we needed more CRU time than this approach permitted.
Solution: To expand our access to the CRU, we worked closely with the UT Center for Clinical and Translational Science, which manages the CRU, and with MHH, who provides the CRU space. After many

meetings, phone calls, and emails, we finally received permission for our MHH-credentialed Research Nurses to use the CRU space and resources after hours (i.e. evenings and week-ends) without requiring that a CRU-employed nurse also be present. This was also accomplished in time for us to recruit for the Memorial Day week-end and has been used to good effect since then.

Problem: Since the Clinical Working Group decided that only subjects with a documented BAL could be enrolled, as a temporary measure until we could add a portable breathalyzer test to our protocol, we were dependent upon each recruitment site's policy regarding this test. This requirement eliminated all potential subjects at MHH, as it is their practice to obtain BALs only when medically necessary and mild TBI or minor extremity injury did not meet their standard for this. At BTGH, which has a community alcohol treatment program and views it as their duty to screen for this, we were able to obtain BALs. This was true for the EC, but not the Ortho Emergency Clinic, where the majority of minor extremity trauma patients are treated. It was not possible to get a BAL for these potential candidates, either. The result was that the first 6 subjects were MTBI patients enrolled from the BTGH EC, and no subjects were enrolled at MHH until after the breathalyzer amendment was approved in mid-May.
Solution: We submitted a protocol amendment, approved in May 2010, to conduct a breathalyzer test after enrollment to determine if the subject had alcohol in their system and if so, how much there was. Since then, there have been 5 subjects enrolled at MHH (2 MTBI, 3 Ortho), and 1 Ortho subject from the Ortho Emergency Clinic at BTGH. Of the 10 subjects enrolled in May, June, and July, we would have missed these 5 subjects without the breathalyzer capability.

Problem: We developed our operational guidelines for the Integrated Clinical Protocol on the assumption that subjects would prefer to complete Baseline Visit activities immediately after their EC visit. Since our protocol includes a 1 week follow-up visit, our assumption was that subjects would not want to go home after the EC visit, return for Baseline Visit activities within 24 hours of injury, and then return again in 7 days. However, we have discovered that relatively few people are willing to spend an additional 4-6 hours in research activities after spending 4-12 hours in the EC to be treated for their injuries. The most frequent exclusion criterion that we are using is "Not Interested" which accounts for 33% of excluded subjects. The reason given for the "Not Interested" response is the time required. For example, during the Memorial Day week-end (May 28-31), our Research Nurses screened 13 subjects that had no exclusion criteria, yet none were willing to stay and participate in our research, so they were excluded as "Not Interested". A full discussion of screened and excluded subjects is presented in Dr. Levin's report.
Solution: We have modified our study operational routines so that a subject may be enrolled during their EC visit, go home, and then return within the 24 hour window for the Baseline Visit activities (see Figure 2 below).

Figure 2: Baseline Visit Options

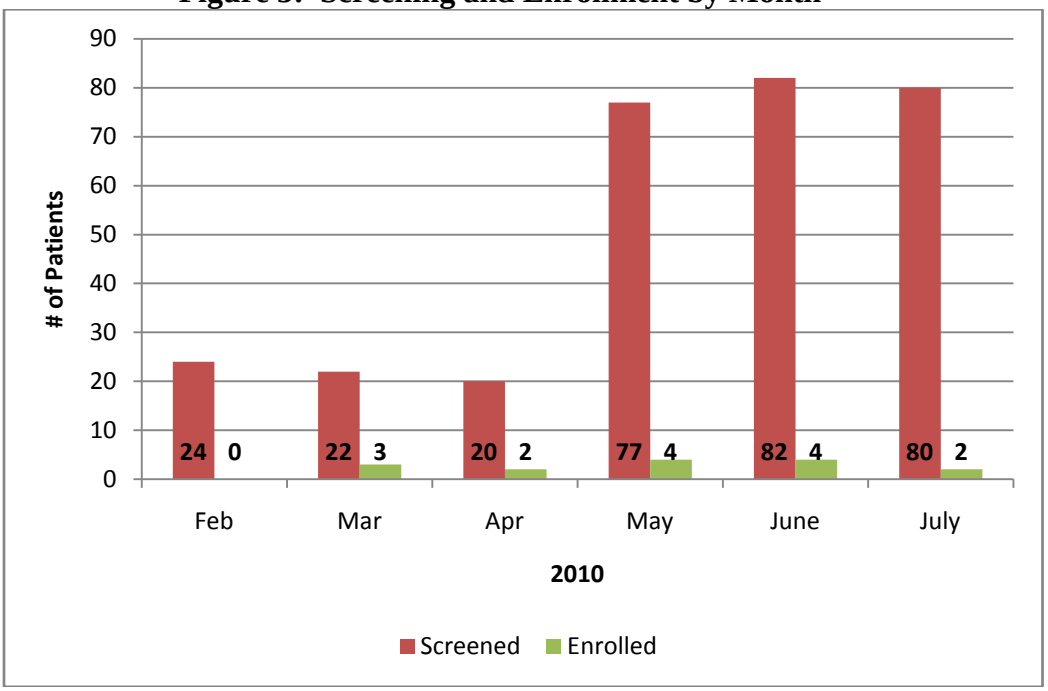


For example, one subject was injured around 3 PM, spent 5:00-10:30 PM in the EC, and was identified by our Research Nurse through remote computer access. The Research Nurse called the EC and spoke with the potential subject, who was willing to return the next day. On the following day, all study activities, including obtaining informed consent, were completed prior to 3 PM (end of 24 hour window). We have done this now for 4 subjects, that would otherwise have declined to participate. So, this is an option that we now offer, and we are optimistic that it will help accrual.

Solution: Subjects currently receive $125 in compensation for their time for the Baseline Visit, part of a total of $475 paid over the course of the 6-month study protocol. We have submitted a protocol amendment to increase this compensation amount to $200 for the Baseline Visit, an increase of $75 that would change the 6-month total compensation amount to $550. We hope that this will increase the number of subjects willing to enroll in the subject. A budget revision has also been submitted to CDRMP.

Since the institution of these changes by mid-May, our subject enrollment has increased significantly. In the time between start of enrollment (Feb 4) and the end of April (86 days), we screened 66 candidates, and 5 of these were enrolled. From May through July (92 days), we screened 239 candidates and enrolled 10, essentially tripling our screening efforts and doubling enrollment. The screened and enrolled subjects by month are presented in Figure 3 below:

Figure 3: Screening and Enrollment by Month



In addition to screening and recruitment, the Clinical Working Group also plays a key role in monitoring protocol compliance and operational performance. There is regular discussion of protocol activities, issues, and concerns in the regular meetings of the Clinical Working Group. Minutes of these meetings are taken and available on the Consortium web-site. All decisions regarding protocol procedures and amendments are handled through this group. We are monitoring compliance with the inclusion and exclusion criteria (discussed in detail in Dr. Levin's report), timeliness of return visits, correct execution of study procedures, and adverse events.

To date, all return visits except 1 (subject had family emergency) have been conducted within the designate time window. When the automated data entry and reporting system is operational, reports will be

generated as to the timeliness and quality of study procedures. We have had some difficulty with the EEG protocol at Memorial Hermann, which has since been resolved (see Dr. Mizrahi's report for further discussion of this). There have been 2 subjects that had incidental findings on the Baseline MRI. Neither was interpreted as requiring immediate medical intervention or as constituting reason for withdrawal from the study. Dr. Claudia Robertson met with each subject to review the findings and recommend that they consult their personal physician, and the subjects were given a CD copy of their MRI.

There have been no Severe Adverse Events (SAEs) thus far. We have recorded 6 Adverse Events (AEs). Four of these were procedural, related to the timing of study procedures (e.g. discovered after enrollment that subject had a hair weave that prohibited EEG electrode placement; subject subsequently had weave removed and Baseline EEG was obtained at the 1 Week visit). Two AEs were recorded for 2 subjects enrolled in the medication trial. One subject reported gastric upset at the 1 Week visit, but had not done so on the Day 3-4 phone call, and in fact had completed all 7 doses of medication. A second subject reported fatigue and gastric upset with diarrhea, stopped taking the study medication, and reported this to the Research Nurse on the Day 3-4 phone call. All of these symptoms are known to be associated with the study drug, atorvastatin, and they are listed in the Informed Consent document.

In this second year of the project, the Clinical Working Group has monitored procedures for screening and enrolling subjects in the Integrated Clinical Protocol. Although it has been necessary to evaluate and revise our original plans in some areas, overall, this process is working well. After overcoming a number of obstacles to subject accrual, we have revised our procedures with a significant increase in enrollment rate and anticipate that this will continue to increase. We are currently using manual versions of our case report forms (CRFs) for data collection, but the development of our automated data system is moving along rapidly, and we anticipate a fully automated process by mid-Fall of 2010.

Collaboration with Other DoD-funded Investigators The Clinical Working Group is finalizing plans to implement an addition to our protocol that reflects a collaborative effort with Dr. Jam Ghajar. Dr. Ghajar is developing an eye tracking test as a diagnostic tool for mild TBI. Our group had several conference calls with Dr. Ghajar, and he came to Houston and made a presentation about this test and the device used to accomplish it. We plan to test this device on our subjects as well, thanks to the additional funding for this purpose. Since we are conducting our initial evaluation of subjects within 24 hours of injury, this will provide valuable data about the usefulness and applicability of this test. We have purchased the equipment and plan to begin training of our research staff as soon as it arrives.

Military Relevance: Data and Results The goal of Specific Aim 2.0 is to improve the diagnosis of mild TBI, developing more objective criteria in the early post-injury period, as well as in the chronic condition. To meet this objective, we are enrolling civilian subjects within 24 hours of injury and conducting detailed evaluations of their condition after injury through their participation in three observational studies (Specific Aims 2.1 – Levin PI, 2.2 – Papanicolaou PI, and 2.3 – Masel PI), where detailed data are collected using cognitive/behavioral techniques, EEG, MRI, and MEG over a six month period. To ensure that the data and subsequent results are meaningful to the mild TBI experience in the military setting, we have included assessments/evaluations used by/relevant to the military (i.e. the Military Acute Concussion Evaluation, the ANAM) that will permit us to compare our subject group to military populations.

The Automated Neuropsychological Assessment Metrics (ANAM) Battery was selected for this project to enhance relevance to military application. The ANAM was developed to address the challenges of assessing cognitive performance during complex operations in extreme and hazardous environments, and is currently being used in the Army to evaluate mild traumatic brain injury. It allows for the mapping of validated laboratory based metrics onto individual military performance.[6,7,8] During its bi-weekly meeting held

March 16, 2010, The Clinical Working Group held a very productive video conference with LTC Michael L. Russell, who is the Director of the Automated Neuropsychological Assessment Metric (ANAM) program for the Department of the Army (HQ, USA MEDCOM). At the suggestion of the Army Surgeons General office, Dr Holcomb invited him to present his experience using the ANAM which is currently used *in theater* by the military and shared some data and current findings from the use of the ANAM in service members deployed in Iraq and Afghanistan. Our group presented our experiences thus far with the ANAM. The Clinical Working Group is currently discussing additional military resources that we may seek out for further collaboration and consultation.

In order to assess the comparability of our civilian sample with military cohorts with mild TBI, we have included the Military Acute Concussion Evaluation (MACE) to our test battery. The MACE is currently used by medics and other medical personnel to assess soldiers for the presence of concussion/mild TBI in Iraq and Afghanistan; it is the only standardized and most widely used method for evaluating acute mild TBI in military operational settings.[9] There are only two published reports of its use thus far, and both suggest that this tool may have limited utility, particularly if administered more than 12 hours after the mild TBI.[10, 11] While we recognize these limitations, our subjects will receive their initial MACE within 24 hours and a significant portion of these will be within 12 hours. We hope that the generalizability of our findings, in terms of sequelae and outcome, of our civilian sample relative to military populations will be improved with our ability to demonstrate the comparability of MACE scores from active duty settings.

Military Relevance: Consultation with Military Clinicians Drawing upon Dr. Holcomb's contacts and recommendations from the EAB, we have in recent months established a network for military clinicians that act as a "sounding board" for us in implementing our Integrated Clinical Protocol. These interactions range from email/telephone information exchanges to video conferences, such as we had with LTC Michael L. Russell, the ANAM Director, described above. To date, we have established consultative relationships with:

- Major Jeffrey Lewis, MD, PhD, USAF, MC, Bethesda, MD
- CAPT Mike Hoffer, San Diego Naval Med CTR
- COL Heidi Terrio, Chief, SRP Center, Ft Carson CO
- LTC Michael L. Russell, ANAM Program, Alexandria, VA

We are in the process of scheduling video conferences with some of these experts and our Clinical Working Group for several of our fall meetings, and we look forward to their input.

Summary Reports for Consortium Projects

Project Summaries by Specific Aim
August 2010

(NOTE: Projects in the Integrated Clinical Protocol are designated with *)

Specific Aim #1. **To characterize animal models of mild traumatic brain injury (MTBI) in order to mimic the neurobehavioral deficits observed in human MTBI patients.**

Specific Aim #1.0.

Investigators

D. DeWitt, PhD – PI
C. Robertson, MD
P. Dash, PhD
P. Narayana, PhD
R. Grill, PhD
J. Holcomb, MD

Project Summary

Introduction: Basic research designed to improve the diagnosis and outcome after MTBI requires the use of reproducible animal models that replicate important patho-physiological features of TBI in patients. Any of the widely used experimental TBI models can be modified to cause mild injury, but typically the clinical features of MTBI have not been assessed. In addition, there are few well-characterized models of blast-induced brain injury (BIBI) that isolate the effects of blast to the brain rather than the body. The goal of this section of the project is to thoroughly characterize the physiological, histological, cerebral vascular and behavioral effects of mild fluid percussion injury alone and in combination with hemorrhagic hypotension and BIBI.

Progress: To explore the effects of blast neuronal, glial and cerebral vascular injury, BBB permeability and cellular immune responses, immunohistochemical staining was performed using brain tissue samples harvested 24 - 72 hrs after mild-moderate levels of BIBI. These experiments are summarized in the table:

Purpose of IHC Staining	Protein Stained	Results
BBB permeability	IGg Albumin	↑ IGg & albumin staining = bilateral, midline cortical BBB damage
Dendritic injury	MAP-2	↓ MAP-2 = bilateral dendritic injury
Blood vessel Inflammation	Vimentin	↑Vimentin = inflammation of cortical & hippocampal blood vessels
blood vessel injury	Collagen IV	↑ Collagen IV = vascular injury in cortex & hippocampus
Neuronal injury & recovery	BDNF	↑ BDNF = midline cortical neuronal injury
Astrocyte injury	GFAP	↓ GFAP = Hippocampal astrocytic damage
Cellular immune system activation	Myeloperoxidase CD68	↑ Myeloperoxidase & CD68 = midline neutrophil & macrophage activation, respectively
ONOO formation	Nitrotyrosine	↑ Nitrotyrosine = ONOO formation in cortex & hippocampus
All IHC staining performed at 24 hrs. post-blast except GFAP at 72 hrs.		
BBB – blood-brain barrier; BDNF – brain derived neurotrophic factor; GFAP - glial fibrillary acidic protein; IHC – immunohistochemical; MAP – microtubule associated protein; ONOO - peroxynitrite		

In rats subjected to Sham injury, very little staining of albumin (green) or IgG (red) was observed. In contrast, rats subjected to moderate BIBI exhibited marked staining for both proteins in the midline superior cortex. To determine the effects of BIBI on astrocytes, sections from brains harvested from rats seventy-two hours after blast or sham injury were stained for GFAP, a marker of astrocytic cytoskeletal proteins. Rats subjected to

<table>
<tr><td></td><td></td><td>moderate BIBI exhibited reduced GFAP staining. In addition to the apparent GFAP downregulation, hippocampal astrocytes exhibited disordered morphology, suggesting blast-induced astrocytic cytoskeletal injury. To determine the effects of BIBI on cellular immune responses, sections from brains harvested from rats seventy-two hours after blast or sham injury were stained for myeloperoxidase, an indicator of activated neutrophils or CD68-IR, an indicator of macrohage activiation. Twenty-four hours after moderate BIBI, increased immunoreactivity for myeloperoxidase (red) and CD68 (green) were observed in the midline cerebral cortex of rats subjected to BIBI. To determine whether blast injury was associated with the production of peroxynitrite, sections harvested from brains of rats subjected to moderate BIBI were stained with antibodies for nitrotyrosine. In sham-injured rats, very little nitrotyrosine staining was observed. However, twenty-four hours after BIBI, nitrotyrosine immunoreactvity (brown precipitate) was observed in the cerebral cortex and hippocampus.
Conclusion: In summary, these studies indicate that BIBI results in dendritic injury (reduced MAP-2 staining), cerebral vascular injury (increased vimentin and collagen IV staining), damage to the blood-brain barrier (increased extravascular albumin and IgG staining) and astrocytic activation (increased vimentin staining). In addition, BIBI is followed by astrocytic injury (GFAP staining), neurotrophil and macrophage activation and migration to the injured cortex (myeloperoxidase and CD68 staining) and increased peroxynitrite production (increased nitrotyrosine immunoreactivity in the injured cortex and hippocampus. These studies are a result of collaborations with Drs. Raymond Grill (University of Texas Health Sciences Center at Houston) and Pramod Dash (University of Texas Health Sciences Center at Houston). The modifications planned for our current blast device as well as the new devices produced by David Ritzel and Steven Parks will result in a unique facility capable of studying the effects of blast waves generated by explosive combustibles (the current Vandenberg model), high pressure helium and a combustible oxyacetylene gas mixture.</td></tr>
<tr><td colspan="3">***Specific Aim #2:* To improve the diagnosis of MTBI, developing more objective criteria in the early post injury period as well as in the chronic condition.**</td></tr>
<tr><td colspan="3">***Specific Aim #2.1:* To study early (<24 hr) diagnosis of MTBI and differentiation of its sequelae related to brain injury from PTSD symptoms present in MTBI and in orthopedic injury (OI) groups studied over a six month follow-up interval. (Includes the Neuropsychology Core)**</td></tr>
<tr><td>***- #2.1.1*** To investigate differentiation of patients sustaining MTBI from a group of patients with OI based on cognitive performance, diffusion tensor imaging (DTI), and EEG findings within 24 hours after injury and changes within DTI, EEG, and cognitive performance over six months following injury.</td><td rowspan="2">Investigators
H. Levin, PhD -- PI
S. McCauley, PhD
L. Wilde, PhD
J. Hunter, MD
G. Hanten, PhD</td><td rowspan="2">Project Summary
This report describes the progress for Specific Aim 2.1, which focuses on analysis of early (< 24 hours) MTBI and differentiation of brain injury sequelae from post-traumatic stress disorder (PTSD) symptoms and the Neuropsychology Core, which is responsible for subject recruitment and follow-up for all the clinical projects in the Integrated Clinical Protocol, as well as conducting all neuropsychological testing.

SPECIFIC AIM 2.1 Dr. Levin's work in this reporting period has been directed toward three specific objectives:
1. Differentiation of MTBI from non-brain injury trauma on cognitive performance, DTI, and EEG
2. Differentiation of PCS and ASD after brain injury and orthopedic injury
3. Identification changes in cognitive performance, PCS, PTSD symptoms, depressive symptoms, and functional outcome
These objectives will be accomplished through analysis of data collected from subjects participating in the Integrated Clinical Protocol. As yet, only 15 subjects (9 MTBI and 6 Ortho Injury) have been enrolled, so this analysis has not yet begun.

NEUROPSYCHOLOGY CORE The Neuropsychology Core is responsible for subject recruitment and follow-up for all the clinical projects in the Integrated Clinical Protocol, as well as conducting all neuropsychological testing. The Clinical Working Group continues to be an active and effective group for problem-solving and on-going management of the Integrated Clinical Protocol. All required continuing reviews for the BCM and UT IRBs,</td></tr>
<tr><td>***- #2.1.2*** To investigate changes in acute stress disorder (ASD) symptoms and acute post-concussion</td></tr>
</table>

symptoms (PCS) in relation to DTI and EEG in groups of patients with MTIB or OI studied within 24 hours, at 7 days, and at 1 month following injury. - ***#2.1.3*** To investigate changes in cognitive performance, PC, PTSD symptoms, depressive symptoms, and functional outcome over six months following MTBI or OI.		institutional approvals from BTGH and MHH, and HRPO have been completed and are up-to-date. During this reporting period, activities of the Neuropsychology Core have been directed toward: **Subject Screening and Enrollment** We present here the screening and enrollment data for the Integrated Clinical Protocol through July 31, 2010. Currently, 320 potential subjects have been screened by study personnel, with 305 excluded and 15 enrolled. Of the 15 enrolled subjects, 9 (60%) are MTBI subjects enrolled in All Studies (n=5, 33%) or in the Testing/Imaging Studies (n=4, 27%) only. Six are Orthopedic Injury control subjects (40%). The original plan for subject enrollment was a case-finding approach where Research Nurses were stationed during "peak hours" in the Emergency Centers (ECs) of the two enrollment sites, Ben Taub General Hospital (BTGH) and Memorial Hermann Hospital (MHH). Our assumption that we could accrue an adequate number of subject using this approach was not correct; therefore, procedures for screening/enrolling subjects have been revised, based on our experience and screening data, so that we now run a 24 hour/7 day on-call schedule rather than using targeted screening times. In addition, we have negotiated full access to the Clinical Research Unit after-hours (i.e. all evenings, week-ends and holidays). We are now offering two options now for subjects to complete Baseline Visit activities: 1) they may complete them immediately upon leaving the EC, or 2) they can go home after they EC visit, returning for Baseline procedures within 24 hours of their time of injury. This was necessary because so many potential subjects did not want to stay for study activities after their long EC visit for their injury. The requirement to have a documented blood alcohol level (BAL) in this population where it is not routinely done for medical reasons has been removed through the addition of a protocol amendment to use a breathalyzer evaluation. This has greatly expanded the population of available subjects. **Prior to these changes, we enrolled 5 subjects over a 3 month period. After their implementation, we have enrolled 10 subjects over the same time period.** **Case Report Forms** Case report forms (CRFs) have been developed based on data/variable lists reviewed and approved by the Clinical Working Group. We are currently testing these forms in "hard-copy" format and programming of them into an automated data collection system is currently underway by our data management service, Silverwind Research. **Electronic Case Report Forms for Neuropsychological Test Battery** Each page of the CRFs has been checked and received signature approval by Dr. McCauley and Dr. Levin before being transmitted to Silverwind Research, our data management service, for conversion into electronic CRFs. These CRFs are then compiled into encounter-specific groupings (i.e., all 1-week tests, all 3-month tests, etc). The electronic CRFs present the tests in a predetermined order and alert the Research Coordinators when to administer delayed memory recall measures thereby minimizing data loss. The CRFs have been carefully designed to minimize data entry errors with range checks and other similar features. **Research Staff Organization and Training** We currently have two full-time Research Nurses and two Research Coordinators III. Training to perform the protocol's neuropsychological test battery for the Research Nurses (Baseline Visit tests only) and Research Coordinators (all tests) has been completed. The Outcome Monitor (Dr. McCauley) has verified accurate administration of the screening measures by the Research Nurses. The Outcome Monitor also has verified accurate administration of the psychological and neuropsychological

		outcome measures by the Research Coordinators. **Data Quality Control for the Neuropsychological Testing Procedures and Data** Monitoring of Data Collection and Data Quality Control is on-going. Inter-rater agreement on neuropsychological tests with subjective scoring has been achieved at $\geq$ 95%.The first three 3-month assessments (an encounter which incorporates all psychological and neuropsychological outcome measures) have been observed by the Outcome Monitor with no deviations from standard administration. The accuracy of the scoring of these tests has been verified as correct and accurate. The audio recordings of the first three CAPS interviews have been reviewed by the Outcome Monitor with no deviations from standard administration; the Research Coordinators' diagnostic classification for PTSD was verified as accurate. **Imaging Data Acquisition, Transfer and Analysis** Operational procedures for the acquisition and transfer of data have been established and followed, resulting in imaging data having been acquired on all enrolled participants at the initial time point as well as all participants who have exited their 3-month window. Data transfer has been performed in a timely fashion, allowing expedited review by the project neuroradiologist for any incidental findings and review by Drs. Wilde and Hunter for image quality. Dr. Wilde has overseen the analysis of volumetric, diffusion tensor analysis and magnetization transfer imaging data, which has been completed for data in these modalities. Data quality has been excellent, and there were no difficulties in analysis. **Conclusion** In this second year of the project, we have initiated procedures for screening and enrolling subjects. Although it has been necessary to evaluate and revise our original plans in some areas, overall, this process is working well. After overcoming a number of obstacles to subject accrual, we have revised our procedures with a significant increase in enrollment rate and anticipate that this will continue to increase. The development and testing of CRFs for data collection is currently in manual form, but the development of our automated data system is moving along rapidly, and we anticipate a fully automated process by mid-Fall of 2010.
***Specific aim #2.2:* To study late (>48hr) diagnosis of MTBI and differentiation of MTBI and PTSD**		
- #2.2.1 To develop more sensitive diagnostic tests for MTBI: 1. using MRI imaging techniques including high resolution MRI, DTI, susceptibility-weighted MRI, magnetic resonance spectroscopy (MRS) and perfusion imaging, and 2. using magneto-	**Investigators** A. Papanicolaou, PhD – PI J. Breier, PhD E. Castillo, PhD T. Kent, MD	**Project Summary** **Introduction**: The aim of this proposal is to detect and characterize focal abnormalities in neurophysiological function in patients with mTBI and PTSD using magnetoencephalography (MEG) for the purpose of distinguishing between the two. We also propose to explore the relationship between diffusion tensor imaging (DTI) and MEG findings. While MEG provides data regarding focal abnormalities in neural response in the cortex, DTI reveals the status of white matter tracts that form the intracortical connections. Thus, MEG, in combination with DTI, may lead to identification of more distinct, replicable patterns of brain abnormalities in subjects with PTSD and mTBI that may lead to better differentiation between these groups of patients, as well as from patients with a combination of both disorders. **Progress**: As of July 31, 15 subjects have been enrolled, with 4 subjects completing the Three Month follow-up visit when the MEG is performed. All visits were performed within the designated time window, and the MEG

encephalography (MEG)		procedures have gone smoothly In addition, we have participated in collaborative activities with the other investigators on this and the other clinical projects in the Integrated Clinical Protocol and participated in regular meetings of the Clinical Working Group, which monitors and ultimately directs the Integrated Clinical Protocol. **Conclusion**: As of July 31, 2010, 15 subjects have been enrolled and 4 have completed the MEG procedures. Please see Dr. Levin's report (Specific Aim 2.1) which addresses the recruitment and data collection procedures used by the Integrated Clinical Protocol. His report also deals with the fact that recruitment has been slower than anticipated and the measures taken to improve upon this problem.
- ***#2.2.2*** To study the neural basis of the pathology of MTBI by correlating findings with functional MRI, DTI-based tractology, MEG, and neurobehavioral assessments		
- ***#2.2.3*** To study the difference in the underlying patho-phyiology between MTBI and PTSD using functional MRI, DTI-based tractology, and neurobehavioral assessments		
****Specific Aim #2.3:*** **To study diagnosis of post-traumatic hypopituitarism after MTBI**		
- ***#2.3.1*** To determine the incidence of hypopituitarism following MTBI	**Investigators** B. Masel, MD – PI R. Urban, MD	**Project Summary** **Introduction:** The purpose of this project will be to study the diagnosis of post traumatic hypopituitarism after MTBI. We will determine the incidence of hypopituitarism following MTBI and develop criteria for assessing which MTBI patients are at high risk for developing posttraumatic hypopituitarism and should have routine post-injury screening. We will also determine the relationship between post-traumatic hypopituitarism and functional outcome, cognitive recovery, and resolution of PCS at six months after MTBI. **Progress**: Fifteen subjects have been recruited as of July 31, 2010; however, none have reached the 6 month point, where data collection (i.e. blood samples) for this project occurs. I have been an active participant in the Clinical Working Group as well as at the Partnering PI Quarterly meetings. My facility, the Transitional Learning Center in Galveston, TX, holds an invited conference every year. The focus of this year's conference was Translational Research, with the main focus on blast--from the physics to the clinical issues. Many of the speakers and attendees were members of the Mission Connect consortium. A new and separate proposal was submitted to the DOD on March 31, 2010. This proposed study would entail screening and treating blast and non-blast injured soldiers with mild TBI recruited from the Neurology Clinic at Camp Lejeune for pituitary dysfunction. These individuals would be invited to participate in this placebo controlled double blind study of growth hormone replacement. This study would include imaging, physical testing and neuropsychological testing. This study would also include a cohort recruited from the Mission Connect MTBI clinical study. These individuals would have already been screened for pituitary dysfunction and would be invited to participate as well. Many of the investigators in this study are part of the Mission Connect MTBI Consortium. **Conclusion:** As of July 31, 2010, 15 subjects have been recruited for the clinical trials. Dr. Levin's report (Specific Aim 2.1) addresses the recruitment and data collection procedures used by the Integrated Clinical Protocol. This report also deals with the fact that recruitment has been slower than anticipated and the measures taken to improve upon this problem.
- ***#2.3.2*** To develop criteria for assessing which MTBI patients are at high risk for developing post-traumatic hypopituitarism and should have routine post-injury screening.		
- ***#2.3.3*** To determine the relationship between post-traumatic hypopituitarism and functional outcome, cognitive recovery, and resolution of PCS at six months after MTBI		
Specific Aim #3: **To develop new and innovative treatment strategies for MTBI and provide the preclinical and phase 1-2 testing of treatments found to improve outcome.**		

<table>
<tr><td colspan="3">Specific aim #3.1: To study neuroprotection and enhanced neurological recovery with erythropoietin (Epo) and Epo derivatives after MTBI.</td></tr>
<tr><td>- #3.1.1 To study the effects of Epo and Epo derivatives on neurogenesis, angiogenesis, and outcome after experimental MTBI, experimental PTSD, and on experimental MTBI associated with secondary ischemic insults.</td><td>Investigators
C. Robertson, MD – PI
L. Cherian Matthew, PhD</td><td>Project Summary
Introduction: The goal of this project is to study effects of erythropoietin (Epo) and the Epo derivatives carbamylated Epo (CEpo) and ARA290 on neurogenesis, angiogenesis, and outcome from after experimental MTBI, experimental PTSD, and on experimental MTBI complicated by hemorrhagic shock. In year 2, the mild TBI complicated by hemorrhagic shock model developed in year 1 has been used to test the neuroprotective effects of Epo and an Epo derivative (ARA290), which is manufactured by Warren Pharmaceuticals, including a set of animals to study the acute hemodynamic effects of the drugs, and a second set of animals to study the long term neurobehavioral and histological effects of the drugs.
Progress: We are evaluating the effects of erythropoietin (Epo) and the Epo derivative ARA290 on outcome in the mild TBI model developed in Specific Aim #1.5. These studies should be completed within the next 2 months. The early results suggest marked reduction in contusion volume with both drugs, and possibly some earlier neurobehavioral recovery, especially with the ARA290. Preliminary analysis of data from the hemodynamic studies suggests that the blood pressure recovers more completely following resuscitation in the animals that received the active drugs Epo and ARA290. ICP may also be slightly higher with the drug treatment. With ARA290, the blood flow at the core injury site and also the penumbra site appears to be higher.</td></tr>
<tr><td>* -#3.1.2 To confirm that atorvastatin (see note below) given during the acute phase of MTBI has no adverse effects in patients with MTBI
NOTE: Due to an FDA hold on all human studies involving erythropoietin, the neuroprotective agent for this phase II clinical trial was changed to atorvastatin.</td><td rowspan="3">Investigators
C. Robertson, MD – PI
H. Levin, PhD
A. Papanicolaou, PhD
P. Narayana, PhD
P. Swank
J. McCarthy, MD</td><td rowspan="3">Project Summary
Introduction: The Integrated Clinical Protocol includes three observational studies (Specific Aims 2.1, 2.2, 2.3) and one interventional study (Specific Aim 3.1.2-7). This interventional study is a Phase II randomized clinical trial of 200 MTBI subjects to evaluate atorvastatin (Lipitor®) as a neuroprotective agent in the treatment of MTBI. (NOTE: The Ortho Injury (OI) control subjects are not eligible for portion of the Protocol.) The primary outcome measure for the atorvastatin is the Rivermead questionnaire at 3 months post-injury. Subjects are followed for the full 6 months and complete all research procedures in the Protocol.
Progress: As of July 16, 2010, 15 subjects have been enrolled: 9 MTBI and 6 OI. Of the 9 MTBI subjects, 5 (55%) are enrolled in the atorvastatin part of the protocol, and 4 (45%) have declined but are participating in the testing/imaging portion of the protocol. The MTBI patients enrolled in the study are randomly assigned to either a daily dose of atorvastatin of 1mg/kg (up to 80 mg) for seven days and started within 24 hours of MTBI, as compared with a placebo. Subjects, investigators, and study personnel are masked to the treatment group. (Please see Dr. Swank's report for the Statistical Core for further discussion of the randomization plan and procedures.) During the week of study drug treatment, subjects are monitored for adverse events associated with atorvastatin dosage by phone on Day 3 or 4. Then, at each follow-up visit, the MTBI patients are asked about the occurrence of any complications, including constipation, heartburn, stomach gas or pain, allergic reactions like skin rash or itching, swelling of the face or lips or tongue, fever, joint pain, muscle cramps or pain, blistering or peeling inside the mouth, trouble passing urine or change in the amount of urine, or feeling unusually weak or tired. Serum lipid levels and liver function tests are monitored at one week and one month after study drug dosage. To date, all follow-up visits and phone calls in this portion of the protocol have been completed, or are scheduled, within the designated time window. There have been no Severe Adverse Events. Two subjects (2/5, 40%) have reported mild symptoms, such as fatigue and gastric upset, which are known to occur with atorvastatin dosage, and these have been recorded in the study database as Adverse Events.
Conclusion: As of July 16, 2010, 8 MTBI subjects have been enrolled, of 15 total (9 MTBI, 6 OI), and 5 are enrolled in the atorvastatin portion of the protocol. Please see Dr. Levin's report (Specific Aim 2.1) which addresses the recruitment and data collection procedures used by the Integrated Clinical Protocol. This report also deals with the fact that recruitment has been slower than anticipated and the measures taken to improve upon this problem.</td></tr>
<tr><td>* - #3.1.3 To study the effects of atorvastatin given during the acute phase of MTBI on development of PCS, PTSD symptoms, cognitive recovery, and functional outcome over the first six months after injury with three months as the primary endpoint.</td></tr>
<tr><td>* - #3.1.4 To evaluate the effects of atorvastatin on brain region and whole brain volumes measured by MRI data and the integrity</td></tr>
</table>

of white matter tracts using DTI at 3 months post-injury. As a research questions, does DTI acquired in the early phase of recovery provide a biomarker for prognosis and response to atorvastatin?		
**** - #3.1.5*** To assess the effects of atorvastatin on normalization of EEG activity at 3 months after MTBI. As a research question, is the pattern of EEG recorded early after MTBI related to response to atorvastatin?		
**** - #3.1.6*** To investigate the relation of EEG recordings during the acute phase of MTBI to MSI patterns at 3 months and evaluate the predictive utility of early EEG for resolution of post-concussion, PTSD, and depressive symptoms, cognitive deficit, and functional outcome of MTBI at 3 months.		
**** - 3.1.7*** To examine the effects of comorbid injury to body regions other than the head on response to atorvastatin and outcome of MTBI at 3 months as measured by PCS, PTSD, cognitive, and functional outcome.		
Specific aim #3.2: **To target the Wnt-GSK3 signaling pathway for the treatment of cognitive deficits after TBI**		
- 3.2.1 To characterize the temporal and spatial	**Investigators** P. Dash, PhD – PI	**Project Summary** **Introduction:** The Wnt/GSK3□□□ catenin pathway is involved in multiple facets of neural growth and

chances in the canonical Wnt/GSK3β/β-catenin signaling pathway in the hippocampus and cortex after MTBI.	S. Pati, PhD	development and may provide the key to regeneration, repair and plasticity of the adult brain. The primary of objectives of our study is to examine the role of Wnt-GSK-3 pathway in TBI pathophysiology employing two FDA approved drugs (valproate and lithium) in a rodent model of traumatic brain injury (TBI). In addition, the histopathological and behavioral consequences of mild fluid percussion injury (MFPI) will be investigated. During these first two years of funding, we have tested the efficacy of post-injury administration of these drugs on the motor, cognitive, and morphological dysfunctions observed following TBI in rodents. Since the Consortium partners are in the process of characterizing the pathophysiology of mild TBI (TBI) and the MTBI-associated behavioral outcomes are not well defined, we have evaluated the therapeutic efficacy of valproic acid and lithium employing a moderate level of TBI. **Progress**: During the current funding period we have made the following discoveries. • We have found that post-TBI administration of 400 mg/kg valproate significantly reduced TBI-associated blood-brain barrier (BBB) permeability. • We have demonstrated that post-injury administration of 400 mg/kg, but not 100 mg/kg, of valproate offers neuroprotection in moderate-severe brain injured animals. • We have found that 400mg/kg valproate lessens TBI-associated memory dysfunction when administered 30min, but not 3hr, post-injury. • Our evaluation of the consequences of post-injury lithium administration has revealed that 1meQ lithium chloride, when given 30min post-injury, improves cognitive function. **Conclusion**: Our results to date show that valproic acid and lithium, both having multiple targets of action, can be used to reduce the histopathological and behavioral problems associated with TBI. These improvements were not observed when agents that specifically target one mechanism (i.e. HDAC inhibition, GSK-3 inhibition) were used, suggesting that the combination of multiple actions was required for their effects. During the future funding periods, we will continue our evaluation of the beneficial influences of lithium chloride administration and will perform histological analysis of the brains from lithium-treated, injured rats.
- ***#3.2.2*** To determine how modulations of Wnt/GSK3~/~catenin signaling pathway after MTBI with lithium and valproic acid affects hippocampal function and the cognitive deficits which are characteristically found after MTBI		
- ***#3.2.3*** To determine how modulation of GSK-3 after TBI and cognitive changes observed in Specific Aim #3.2.2 correlate to changes in axonal and dendritic morphology, cell death, and neurogenesis after MTBI.		
Specific aim #3.3: **To study the role of IL-1 and TNF receptor activation in neurological deficits after TBI**		
- ***#3.3.1*** To serially measure brain cytokine levels after MTBI	**Investigators** R. Perez-Polo, PhD – PI C. Hulsebosch, PhD P. Dash, PhD D. DeWitt	**Project Summary** **Introduction**: Although mild traumatic brain injury (mTBI) is an important clinical outcome of combat, with long term consequences resulting from attendant cognitive and behavioral deficits, there is a dearth of specific knowledge as to the mechanisms at work and potential clinical interventions. We know that ambient levels of key inflammatory cytokines usually increase after traumatic insults to the brain and that they may play a role in the development of long-term deficits. Although the cytokine increases are but one component of monocyte cellular infiltration, vascular perturbations and persistent inflammation**, it is our global hypothesis that trauma-induced cytokine activity has a role in the long term deficits associated with mTBI**. We have shown that there are also both early increases in IL-1 and TNFα cytokine levels known to contribute to cell death and inflammation, as well as more long term increases in microglial and astrocytic activation, and increased levels of biomarkers associated with vascular dysfunction: all resulting in behavioral and cognitive dysfunction after mTBI in a lateral fluid percussion rodent model of mTBI (1 atm). Our central hypothesis is that **blocking early inflammatory cytokine signaling after mTBI will improve outcomes by ameliorating inflammation and mTBI-associated neurological deficits.** **Progress**: During this last year we screened rats suffering a mLFP injury (1atm) for cellular biomarkers of inflammation that are known to participate in the various pathways that result in neuropathology and impaired neural/vascular function as well as behavioral assays indicative and relevant to both the mLFP injury and the military experience. We also participated and completed assays for "common biomarkers" for all projects as agreed to by the modeling and protection working groups. I chair the protection working group. Finally, we
- ***#3.3.2*** To study the role of IL-1 receptor activation in neuronal cell death and in the inflammatory response after MTBI		
- ***#3.3.3*** To study the role of TNF receptor activation in neurological deficits after TBI		

		carried out our first intervention using Kineret to block IL-1 inflammatory signaling and observed mild improvement in the working memory and beam balance assays after two injections ip of Kineret administered 30min and 6hours after injury. This last finding is important because there was some question by the EAB as to the feasibility of Kineret treatment given it's a large protein in spite of the literature suggesting it can go beyond the BBB and the fact that even mild brain injuries or stresses are known to impair the BBB. This latter is also consistent with our finding vascular impairment to be very significant after mLFP injury – see effects on albumin and SMI-71. We have performed the first Kineret intervention with promising results for the i.p. treatment, an important consideration. Optimization and validation are under way. During the summer, and hopefully prior to August 10th, we plan to finish the immunohistochemical and immunoassay characterization of the effects of Kineret treatment and also start a new series of treatments doubling the dose at 30 minutes and then further injecting single doses of Kineret at 3 and 6 hours and daily until day 10 when behavioral assessments will take place with final sacrifice at day 18 for IHC analyses. In the fall, we plan to write our first paper based on these findings. We presented our work at the meeting of the American Society for Neurochemistry, National Neurotrauma Society, and the Society for Neuroscience. We have also submitted an abstract for presentation at the next Society for Neuroscience meeting in the fall. **Conclusion**: Our overall goal is to develop, characterize and assess interventions that will ameliorate the long term neurological deficits following mTBI by ameliorating various injury-induced acute inflammatory mediators in the injured brain. We will measure brain cytokine/chemokine and inflammatory biomarkers after blocking receptors for key inflammatory cytokines: Kineret for the IL-1 receptor that binds both IL-1α and IL-1β and Etanercept for the TNFα, β receptors after mTBI. We will also measure levels of biomarkers reflecting astrocytic activation and vascular dysfunction after these interventions. Finally, we will perform relevant assessments of behavioral and cognitive function after mTBI treatment with Kineret and Etanercept in this rodent model. These interventions were chosen because of existing literature suggesting their beneficial role in ameliorating consequences of spinal cord injury and perinatal ischemia as well as being FDA-approved with few adverse effects. Thus, they could rapidly be moved into clinical testing if successful in these studies relying on the use of rodent models of mTBI.
Specific aim #3.4: **To study disruption of ion channel clustering after TBI**		
- #3.4.1 To study ion channel loss from nodes and the axon initial segment (AIS) in an *in vitro* injury model and determine if inhibiting proteolysis of cytoskeletal proteins reduces ion channel disruption. ***- #3.4.2.*** To determine if exposure to primary blast injury results in impaired nervous system function using neurobehavioral assays and immune-histochemical evaluations of the structure of axon initial segments (AIS) and	**Investigator** M. Rasband, PhD -- PI	**Project Summary** **Animals:** 6-8 week old male rats will be used for all experiments proposed in Aims 2 and 3. We will use 10 injured and 10 control rats at each time-point, and for each behavioral assay (= 180 rats) and the same number of animals for the experiments proposed in Aim 3 (= 180 rats; this number could decrease depending on the behavioral assays performed in Aim 2). This work will involve a total of 360 rats. **Methods:** **Aim 1: (Completed – see old SOW for a description of the proposed work)** **Aim 2: Part I.** We will perform three behavioral assays to determine if exposure to primary blast injury results in impaired nervous system function. We will use the novel object recognition test (evaluates memory), rotarod (evaluates motor coordination and balance), and fear potentiated startle response (measures anxiety and has been proposed to model components of PTSD). Rats will be tested at 2 days, 1 week, and 2 weeks after blast induced injury. Please note our preliminary data already shows significant alterations at 2 weeks in the novel object recognition test. **Part II.** We will perform immunohistochemical evaluations of the structure of axon initial segments (AIS) and nodes of Ranvier in the blast-injured brain. These experiments are similar to those proposed in AIM3 of the previous SOW, except that we will use our new blast chamber to evaluate a) organization of the cytoskeleton, b) clustering of ion channels, c) gliosis, d) disruption of the blood brain barrier, and e) axon degeneration. We will correlate our results obtained in Part II with those obtained in Part I since we will use the same animals for the

nodes of Ranvier. ***- #3.4.3*** To use the calpain inhibitor MDL28170 to determine if this preserves AIS structure and overall nervous system function after exposure to primary blast injury. These rats will be subjected to the same analyses as proposed in Aim 2 (both behavioral and immunohistochemical stainings).		evaluation of brain structure. Finally, we will extend these experiments to blast-injured brains provided by Dr. Doug Dewitt. **Aim 3:** Based on the observation that we could preserve the structure of the AIS using inhibitors of calpain both in vitro and in vivo (we showed this in Aim 1 of this work and this has been published in Schafer et al., (2009)), we propose for Aim 3 to use the calpain inhibitor MDL28170 to determine if this preserves AIS structure and overall nervous system function. These rats will be subjected to the same analyses as proposed in Aim 2 (both behavioral and immunohistochemical stainings). **Outcomes:** **Aim 1:** We found that AIS are preferentially disrupted after nervous system injury due to activation of calpain, a calcium-dependent protease. Our results are significant since they demonstrate that disruption of the AIS (i.e. loss of clustered ion channels and loss of neuronal polarity) is a previously unappreciated form of nervous system injury. **Aim 2**: We expect to demonstrate conclusively whether blast induced injury disrupts ion channel clustering and if this is correlated with altered behavior. **Aim 3**: We expect to demonstrate whether preservation of the AIS improves nervous system function after blast-induced MTBI.
***Specific Aim #3.5*: To evaluate nanotube delivery of anti-oxidant on neurological recovery following MTBI**		
- #3.5.1 To determine if reduction in oxyradicals by BHT-modified PEGylated CNTs will improve vascular function following MTBI and improve neurological outcome complicated by secondary insults ***- 3.5.2*** To determine if anti-oxidant nanotubes are able to inhibit oxyradical induced epithelial activation and platelet aggregation. ***- #3.5.3*** To determine if modifying the PEG moieties will alter the distribution of antioxidant nanotubes into the brain itself following MTBI.	**Investigators** J. Tour, PhD – PI T. Kent, MD – PI C. Robertson, MD	**Project Summary** Introduction: Oxidative stress is a prominent feature of mild traumatic brain injury (mTBI), in particular when accompanied by secondary insults such as hemorrhagic hypotension. Antioxidant therapies have had limited success in treating mTBI. We identified carbon nanomaterials as potent antioxidants and hypothesize that these materials will be potent antioxidants *in vivo*, with a long duration of action. The mild TBI complicated by hemorrhagic shock model uses a clinically relevant injury + hemorrhage/prehospital care/hospital care paradigm with a time frame that is pertinent to the military setting. Success in this work could be readily translated to the clinic. This belief is based on considerable precedence for approved nanotherapeutics for other conditions and the great potential in the flexibility that these nanotubes provide for combining existing therapeutics with the favorable physiological behavior of the nanotubes. Progress: The work thus far has focused on *in vitro* work that has shown that our nanomaterials are suitable antioxidants to be used *in vivo* for further study in specific aims 3.5.1 and 3.5.3 and preliminary toxicity experiments as they relate to Specific Aim 3.5.2. We have also now begun *in vivo* experiments to address all three specific aims. Dr. Robertson and her lab staff have trained Dr. Kent's assistants in performing the cortical impact injury model and the injury complicated by hemorrhagic shock.This project is a joint effort between Dr. Tour's Nanochemistry laboratory at Rice University and Dr. Kent's oxidative stress, in-vivo and in-vitro laboratories at the Baylor College of Medicine and Michael E. DeBakey VAMC. In addition, whole animal testing is done in collaboration with Dr. Claudia Robertson at the Baylor College of Medicine. For the summaries below, we have designated where the primary effort occurred in parentheses. At the outset of the grant, we (Tour lab) had preliminary evidence that four carbon nanomaterials were promising antioxidants: pluronic wrapped single walled carbon nanotubes (p-SWCNT), PEGylated hydrophilic carbon clusters (PEG-HCC), butylated hydroxytoluene ionically bound to PEGylated hydrophilic carbon clusters (Ionic BHT/PEG-HCC), and BHT covalently bound to PEG-HCCs. Note that hydrophilic carbon clusters are produced from carbon nanotubes (CNTs) and at the time the grant was funded, these materials were identified as CNTs. However, we changed the name to better reflect their structure as the procedure cuts the CNTs into the size range of 30-60 nm (controllable length based upon the cutting temperature ranging from 23 to 70 °C), while destroying the sidewalls and leaving heavily carboxylated addends on these newly generated carbon clusters. In the first year of the grant, we confirmed our preliminary results by performing an oxygen radical absorbency

		capacity assay using a chemical source for the oxygen radical (Tour lab). We published a paper on this subject in the *Journal of the American Chemical Society*. This assay identified the pluronic wrapped SWCNTs and ionic BHT/PEG-HCCs as the two most potent antioxidants. Through the first and second years of this proposal, we (Kent lab) found, after more extensive *in vitro* testing with biologically relevant radicals, that the PEG-HCCs are the superior material due to a combination of their total anti-oxidative absorbance and specific superoxide scavenging activity. Moreover, work occurring in parallel to this proposal has indicated that PEG-HCCs are capable of strongly binding proteins that remain active and can sequester hydrophobic drugs and deliver them *in vivo* (Tour lab). Therefore we proposed to use PEG-HCCs as the basis for further modification to target specific receptors that are expressed in endothelium that will either concentrate the therapeutic nanomaterials at the site of injury (e.g. p-selectin antibody coated antioxidant nanovector (Tour lab)) or facilitate transport across the BBB (e.g. anti-transferrin receptor coated nanovector), or potentially deliver pharmacological agents that can bind onto the nanovectors through hydrophobic interaction. Our results this year indicate that binding of PEG-HCC's with anti-p-selectin antibody dramatically accelerates binding to injured cerebral endothelium in culture, retain their anti-oxidant potential, and protects endothelium from cell death due to inhibition of the mitochondrial electron transport chain (Kent lab). Further toxicity testing indicates no toxicity (Tour lab). Preliminary whole animal work indicates no evident toxicity, while the studies progress at the 6 hour and 24 hour time windows (Kent lab). Two assistants have been trained in the TBI/hypotension/resuscitation procedures (Kent and Robertson labs). Conclusion: The overall aim of this project is to develop novel antioxidant therapies for TBI using nanomaterials that are capable of addressing, for the first time, key components of the "neurovascular unit". If the PEG-HCCs, either targeted or non-targeted, are as effective *in vivo* as they have proven to be *in vitro* they can be envisioned as a new approach to treating mTBI. This technology may allow the flexibility to target specific CNS compartments. Such an achievement will not only allow the testing of important hypotheses, such as the contribution of vascular dysfunction to brain injury, but the possibility of a targeted treatments that ultimately will reduce the likelihood of toxicity while increasing the chances of benefit.
Specific aim #3.6: **To study the use of stem cell-released neurotrophic factor, GDNF, to improve cognitive recovery after TBI**		
- 3.6.1 To determine whether hNSCs grafting into injured brain improves cognitive function following MTBI complicated by a secondary ischemic insult, and whether blocking GDNF eliminates the cognitive improvement. ***- #3.6.2*** To determine whether hNSC-generated GDNF protects host hippocampal neurons following MTBI complicated by secondary insult. ***- #3.6.3*** To determine what GDNF signaling	**Investigators** P. Wu, PhD – PI L. Denner, PhD D. Prough, MD	**Project Summary** **Introduction:** This is the Subproject 3.6 of the Mission Connect Mild TBI Translational Research Consortium. The long-term goal of this project is to characterize and optimize human neural stem cell (hNSCs) transplantation to ameliorate symptoms associated with MTBI followed by hemorrhagic insult, and to enhance post-trauma neurological recovery. **Progress**: Key accomplishments to date include: • The rat mortality rate was reduced from the initial 75% (last year after Ike) down to 16%, which is in the expected range of moderate fluid percussion TBI in rodents. • We discovered that the 2.0 atm moderate fluid percussion TBI plus a secondary ischemic insult did not produce cognitive deficits in rats when examined for their spatial navigation reference memory by the Morris Water Maze (MWM). However, working memory MWM showed trends of impairment in animals after TBI plus ischemia. • Brain tissues of injured animals and controls were collected for further morphological examination. • GDNF neutralizing antibodies were assayed for their bioactivity. • The system of Alzet osmotic pump to infuse GNDF antibodies intrahippocampally was established and validated. • Lentiviral vector-short hairpin RNA was used to knockdown GDNF mRNA expression in human neural stem cells by over 60%.

pathways mediate the in vivo protection effect of hNSC-secreted GDNF following MTBI complicated by a secondary ischemic insult.		• Using an unbiased mass spectrometry approach, we have identified many critical proteins, pathways, and networks dysregulated by TBI, and changed by hNSC transplantation. • Based on the above quantitative proteomics and pathway analysis, we identified and focused on calcineurin proteins that were changed after TBI and cell grafting, and have a known relationship to GDNF signaling pathways. • An in vitro model, rapid stretch injury, was established to mimic TBI in vivo. This model is crucial to further elucidate molecular mechanisms underlying neuronal damage following TBI and the cognitive rescue by NSCs and GDNF-mediated regeneration. • One abstract (#876) is accepted to be presented on the 40th Annual Meeting of the Society for Neuroscience, San Diego, Nov. 2010, and one manuscript is to be submitted soon. **Conclusion**: This project is designed to determine 1) whether human neural stem cell (hNSC) transplantation could prevent cognitive deficits in rats after fluid percussion TBI and a second hemorrhagic insult, and 2) whether and how hNSC-secreted GDNF plays a role in facilitating neuroregeneration. Thus, the rodent model is the key component. As reported in the first annual report, we had experienced unexpectedly high mortality rates in rats. In the last year, working with Dr. Douglas DeWitt, the Animal Care Center at UTMB and ACURO through Dr. Charmaine Richman, we were able to significantly reduce the mortality rate from near 75% down to 16%. The latter is in the normal range of this type of injury. The initial proteomics in combination with the powerful IPA has identified top networks or pathways changed after TBI, which will guide the next step of our studies. When finished, the project will provide evidence for the role of hNSC-secreted GDNF and important insights into molecular mechanisms underlying graft-host interactions that may guide and/or facilitate stem cell or GDNF-aided neural regeneration after MTBI.
Specific aim #3.7: **To study the role of stimulation patterns in eliciting synaptic plasticity**		
- ***#3.7.1*** Determine the contribution of the regularity and pattern of synaptic plasticity of individual neurons in the normal and injured adult rodent neocortex - ***#3.7.2*** Determine the intracellular spatio-temporal dendritic and somatic calcium signals elicited in response to specific temporal patterns of synaptic stimulation in the individual cortical projection neurons of the n normal and injured adult rodent neocorex - ***#3.7.3*** Directly evaluate the sources, contributions, and levels of postsynaptic calcium that are accessed	**Investigator** M. Friedlander, PhD	**Project Summary** **Introduction and Progress:** Towards our goals of determining the most effective combinations of stimulation frequency and temporal pattern of stimuli for the induction of long term changes in synaptic efficiency within the cerebral cortex of the normal and mTBI brain, we have made a number of successful steps in the acute cortical brain slice model of the rat. These include the development of stimulation protocols with regular, highly irregular (Poisson) and intermediate irregular distributions of inter-stimulus intervals at low (1 Hz) and high (10 Hz) stimulation frequencies to quantitatively explore the interactive parameter space of these metrics for the triggering of long term cortical synaptic plasticity; the development and application of a novel discontinuous high frequency (10 Hz) stimulation protocol that prevents synaptic fatigue during conditioning that would override plasticity induction by sustained high frequency (10 Hz) stimulation; and the development and application of a new paradigm that employs low frequency (0.1 Hz) periodic probe synaptic stimulation trials between 10 Hz stimulation periods. This last step allows for the evaluation of the role of the temporal stimulation pattern in the kinetics of synaptic plasticity induction. This would otherwise not be measurable due to the inability to probe the baseline synaptic function during the conditioning protocol. This approach has already demonstrated that there ar5e at least two major types of kinetic profiles of plasticity induction in the control uninjured brain (one linear and one exponential). This protocol will now allow for the evaluation of the contributions of various respective intracellular calcium signals to the establishment of the plasticity. Moreover, by making similar measurements in the mTBI cortex, it should be possible to evaluate the capacity for different individual neurons to most effectively respond to particular synaptic conditioning protocols based on their particular plasticity kinetic profile. This work has recently resulted in two abstracts to be presented as posters at the 2010 annual Society for Neuroscience meeting in San Diego, CA in November, 2010. They are: Fischer, Q., G. Viana di Prisco, G., and

by the synaptic stimulation patterns that are optimal for inducing long term functional changes in synaptic efficiency in individual cortical projection neurons in the normal and injured adult rodent neocortex.		M.J. Friedlander, "Effect of mild traumatic brain injury (mTBI) on induction of synaptic plasticity in rat visual cortex," and Viana di Prisco, G., Q. Fischer and M.J. Friedlander, "Effects of synaptic stimulation pattern on induction of synaptic plasticity in visual cortex." **Project summary:** We have developed and refined stimulation protocols for the systematic exploration of parameter space including frequency with inter stimulus interval regularity for synaptic stimulation of inputs to cortical neurons in an animal model (rat) to allow us to systematically investigate the intersections of frequency and stimulus pattern space in the most effective activation of long term plasticity (long term synaptic potentiation and long term synaptic depression) signaling pathways in normal and mTBI animals. We have effectively ported our earlier preliminary work in the guinea pig brain to the rat. That step was critical for being able to compare the results of our work to a considerable body of literature on TBI in rats from other labs and also to be able to integrate our results with those of the larger research group in our consortium. We have encountered some challenges with respect to the higher frequency stimulation but have overcome them with respect to the discontinuous stimulation protocol. Based on the recommendation of the EAB, we have also been utilizing a standard battery of immunocytochemical analyses of the tissue from the mTBI and control animals to be able to compare the extent of the injuries in our animals to those of others in the consortium who work with rats as well. We meet regularly with this group to discuss our findings and share challenges.
Specific aim #3.8: **To study targeting Rho Family GTPase Signaling Pathways to Enhance Recovery after TBI**		
- #3.8.1 To determine if blocking Rho signaling by pharmacological or genetic means will enhance neuroprotection, neuronal plasticity, and functional recovery after TBI. ***- #3.8.2*** To characterize Rac activation following TBI and determine if enhancing Rac signaling will improve recovery after TBI.	**Investigator** K. Tolias, PhD – PI	**Project Summary** **Introduction**: Cognitive deficits often persist in patients who suffer from TBI. The underlying cause of these impairments involves disruption of neuronal circuits in brain areas important for cognitive function. While some recovery can occur as a result of regenerative processes including axonal and dendritic growth and the formation and remodeling of dendritic spines (sites of excitatory synapses), recovery is often limited due to the hostile growth environment of the adult CNS. A greater understanding of the mechanisms that promote neuronal plasticity and repair is therefore needed for the development of novel therapeutic strategies for treating TBI. A promising approach for enhancing plasticity following TBI involves modulating the activity of Rho GTPases. Rho GTPases play key roles in regulating nervous system development and remodeling. In particular, Rac promotes the growth and branching of axons and dendrites and the formation and maintenance of dendritic spines, whereas RhoA inhibits axonal and dendritic extension and spine growth. RhoA is also robustly activated following both brain and spinal cord injury (SCI), and inhibition of RhoA signaling can results in reduced apoptosis, accelerated regeneration, and enhanced functional recovery after SCI. The goals of our project are to: (1) determine if blocking RhoA signaling will enhance neuroprotection, neuronal plasticity, and/or functional recovery after TBI; and (2) characterize Rac activation following TBI and determine if enhancing Rac signaling will improve recovery after TBI. **Progress**: Key accomplishments to date are: • We have generated a number of genetically modified mouse lines critical for our TBI studies. • We have confirmed loss of RhoA expression in our RhoA conditional knockout (KO) mice (crossed to CamKIIα-Cre and Nestin-Cre) and have begun to characterize these mutant mice. • We identified the Rac activator Tiam1 as a calpain substrate that is degraded after acute neuronal injury caused *in vivo* by TBI and *in vitro* by glutamate-induced excitotoxicity. • We have begun to characterize mice lacking the Rac inhibitors Bcr and/or Abr. We have confirmed elevated Rac activity in the brains of these mice. Furthermore, we have demonstrated that Bcr and Abr play important roles in restricting dendritic spine growth, synapse formation, dendritic arbor elaboration, and astrocyte

		migration. • In collaboration with Dr. Claudia Robertson's laboratory, we have begun to perform TBI experiments on all our mutant mice. Although these studies have proceeded relatively slowly due to personnel changes, we do have some intriguing results. In particular, our preliminary results suggest that inhibition of RhoA in postnatal cortical neurons may have neuroprotective effects against brain injury. We intend to complement these studies with experiments using the pharmacological inhibitor, fasudil. • We have presented our research findings at a number of meetings and universities including a Gordon Conference in Lucca, Italy, the International Society for Neurochemistry meeting in South Korea, the Society for Neuroscience meeting in Chicago, University of Iowa and University of Houston. **Conclusion**: For our first specific aim, we have generated and characterized two different RhoA KO mouse lines, one that appears ideal for investigating RhoA inhibition on recovery from TBI ($RhoA^{flox/flox}$; CamKII□-Cre mice) and another that is useful for determining the role of RhoA in nervous system development ($RhoA^{flox/flox}$; Nestin-Cre mice). For our second specific aim, we have produced several mutant mouse lines (Bcr, Abr, and Bcr/Abr KO mice) that have elevated Rac activity, enabling us to investigate whether Rac activation enhances recovery after TBI. Characterization of these mice indicates that by inhibiting Rac, Bcr and Abr play important roles in restricting the growth of dendritic spines, synapses and dendrites and the migration of astrocytes. In the course of our studies, we have also identified the Rac activator Tiam1 as a calpain substrate that is degraded after acute neuronal injury caused by TBI and glutamate-induced excitotoxicity, suggesting that Rac may normally be inhibited following injury. In collaboration with Dr. Claudia Robertson's laboratory, we have begun to subject all of our mutant mice to TBI experiments to test whether modulating Rho GTPase pathways promotes recovery following TBI. Preliminary experiments suggest that inhibiting RhoA signaling in postnatal cortical and hippocampal neurons may have neuroprotective effects against brain injury, whereas global Rac activation may in some cases hinder repair due to enhanced gliosis. Additional experiments are needed to confirm and clarify these results. Findings from our studies should provide important insight into the role of Rho GTPase signaling pathways in post-injury CNS repair and may help in the development of novel therapeutic strategies to enhance recovery following TBI.
Specific aim #3.9: **To characterize and study rehabilitation of TBI using temporally adaptive functional magnetic resonance imaging**		
- #3.9.1 To study early-stage TBI to examine how improvement in cognitive deficits (which are greatest in the first 6 months) tracks with fMRI-based changes in activation patterns. ***- #3.9.2*** To link clinically diagnostic MR images to unique activation patterns and behavioral measures. ***- #3.9.3*** To develop individualized approaches to fMRI neuro-rehabilitation that consider the unique set of major impairments (and possible concomitant impairments)	**Investigator** S. LaConte, PhD – PI	**Project Summary** **Introduction**: The ultimate goal of this project is to examine the potential of neuro-rehabilitation for mild TBI patients using biofeedback in the real-time functional magnetic resonance imaging (fMRI) system we have developed. In this project, we propose to: 1) increase our understanding of fMRI measurements of plasticity that arise (as evidenced by changes in fMRI-based brain patterns) so that we can better characterize these changes (e.g. are these changes "normalizing" – changing to better match normal activity, or "compensatory" – reorganizing activation by recruiting different brain areas), 2) develop models that link the structural MR images to our knowledge of the patients' cognitive deficits and to the fMRI-based activity, and 3) to develop personalized strategies for neurorehabilitation using real-time whole-brain pattern analysis. **Progress:** We intend to recruit 50 subjects with MTBI and 25 control subjects for this project. The Research Nurses of the Integrated Clinical Protocol (Specific Aims 2.1, 2.2, 2.3, and 3.1.2-7) are actively recruiting subjects for this project, as our inclusion/exclusion criteria will accept subjects that would be excluded from that protocol. As of July 16, 2010, we have received 15 referrals of potential subjects and have enrolled 3. In addition, we have accomplished several important operational goals to support subject enrollment and data analysis. These include: • Hiring and training of personnel to accomplish project outlined in the Statement of Work. • Obtaining HRPO and Baylor College of Medicine IRB approval.

in memory, attention, hemiparesis, mood and anger, or sensory perception.		• Identification and implementation of fMRI stimulus task – the multi-source interference task. • Development of data analysis scripts. • Scientific investigation of spatial transformations of machine learning models of the brain. In addition, we have spent considerable time responding to observations from the External Advisory Board and their requests for further information about this project. The information we prepared (i.e. PowerPoint presentation, written responses) are included in the full report for this project. **Conclusion**: We have assembled and trained a small team of individuals to carry out the project described in the Statement of Work. At this point in time, we have developed the software to present stimuli and to analyze data. In addition, we have HRPO and IRB approval to recruit subjects to complete Aim 1 in year 2 as proposed, and we are coordinating our recruitment activities with the Research Nurses for the Integrated Clinical Protocol.
Specific aim #3.10: **To study inducing plasticity with electrical microstimulation**		
- #3.10.1 To record cortical electrical activity using chronic tetrode arrays while monkeys are learning new tasks. ***- #3.10.*** To determine if electrical micro-stimulation enhances plasticity and learning in area V4. ***- #3.10.3*** To determine if enhancement of plasticity by electrical micro-stimulation of the nucleus basalis induces plasticity across different cortical areas (i.e. it is a widespread phenomenon across the cortex).	**Investigator** A. Tolias, PhD -- PI	**Project Summary** **Introduction:** The long-term goal of the consortium is to improve the diagnosis and treatment of mild traumatic brain injury (MTBI) through collaborative basic and clinical research by experienced TBI investigators. The impact of MTBI on an individual and his/her family can be devastating. These sorts of wounds have become the signature injury in the Iraq and Afghanistan wars and a growing number of our troops are suffering from MTBI. MTBI is a complex injury that is accompanied with a broad spectrum of symptoms and disabilities that vary drastically in severity across individuals. A central issue in MTBI is improvement of sensory and cognitive functions over time. This process of recovery is thought to at least partly rely on learning and plasticity in the adult brain. However, in many cases the improvement is rather limited and in many cases absent. Therefore, understanding the mechanisms of - and ultimately manipulating - learning and plasticity in the brain *in vivo* at the level of circuits and networks of neurons will revolutionize our capabilities to treat these patients. Unfortunately, to date *in vivo* circuit mechanisms of learning and neuroplasticity remain largely elusive. In this project we have two major goals: a) to analyze the *in vivo* mechanisms of neuroplasticity in the cortex and b) to develop methods to enhance neuroplasticity and learning, a promising strategy for delivering a novel paradigm for achieving recovery after brain injury such as MTBI. A better understanding how the brain changes during learning holds great promise to lead to the development of novel treatment strategies that will enhance learning and recovery after MTBI. **Progress in the last year:** a) We have built an infrared eye tracking system for primate research. This provides an additional system to our scleral search coil system that precisely monitors eye movements in monkeys with excellent spatio-temporal resolution. Ongoing comparison tests between the scleral search coil system and our custom build infrared system show that their spatiotemporal resolutions are comparable. Please note that commercially available systems that we tested over the last year were worse in performance to our scleral search coil systems. Therefore, our custom build infrared system allows us to train these animals in different learning tasks (a key component) of our proposal (SA3.10.1, 3.10.2 and 3.10.3) even if the scleral search coil systems fail. b) We have developed an advanced electrophysiological platform that will enable to add a microstimulation module. This new platform will enable us to have the three hardware/software components needed for SA3.10.2 to be integrated together. Specifically, we will be able to simultaneously and in synchronized mode record the activity from neuronal populations, monitor behavioral learning and plasticity and apply microstimulation to enhance plasticity, which is our ultimate goal. c) We have further improved our chronic recording chamber designs. We are happy to report that we have a monkey with a chronic implant for many months now with no obvious signs of infection. We will

<table>
<tr><td></td><td></td><td>know more information after we remove the implants.
d) In the past year we have implanted several monkeys and recorded data from area V4 (SA3.10.1) during learning paradigms. During this time we have collected data to identify the basic rules of how neurons, and which neurons, change their properties during learning. Intriguingly, our preliminary data suggest that neurons in the supragranular layers of the cortex are the ones that seem to be more plastic. These layers of the gray matter of the cortex are the ones that receive input from feedback connections from higher-level areas in the cortical hierarchy. This preliminary discovery will be useful in developing methods to manipulate plasticity after brain injury in MTBI. For example, pharmacological agents, and methods (like microstimulation) which aim to alter feedback inputs and change synaptic connections in the superficial layers of the cortex may be promising for delivering novel therapies for MTBI.
e) We have been working in the optimization of the electrical stimulation parameters, including optimal electrode geometries, which can be used to activate the Nucleus Basalis (SA3.10.2.). We have done simulations to analyze the electrode geometries that would activate initially the whole NB. Please note that in primates the NB is a structure that is around 1cm in diameter. We are investigating with modeling work different electrode arrangements ranging from purchasing the Medtronic deep brain stimulating electrode currently used in Parkinson's patient to designs where we will use custom build electrodes constructed out of Iridium that we have previously developed.
f) We have also begun work and collected data from other areas of the cortex (e.g. area V2) to establish the generality of the principles of plasticity across the different cortical areas (SA3.10.3).
Scientific Conclusions: In the last year we have studied the *in vivo* mechanisms of neuroplasticity in macaques during learning paradigms. Preliminary evidence suggests that the superficial layers in the extrastriate cortex are plastic and the responses of neurons in these layers in the cortex are modified during learning. Thus therapeutic strategies which enhance plasticity in the superficial layers provide a promising target to increase recovery after brain MTBI.</td></tr>
<tr><td colspan="3">***Specific aim #3.11:*** **To develop a macaque fMRI paradigm for studying the mechanism of cortical reorganization after injury**</td></tr>
<tr><td>***- #3.11.1*** To determine whether a commonly used visual rehabilitation strategy enhances adaptive re-organization in higher visual areas thereby improving visual function.
- 3.11.2 To study whether electrical stimulation of the nucleus basalis of Meynert performed in conjunction with visual rehabilitation training enhances adaptive reorganization of visual cortical networks, further improving performance.</td><td>Investigator
S. Smirnakis, MD, PhD – PI
A. Tolias, PhD</td><td>Project Summary
Introduction: TBI frequently results in significant visual impairment as a result of direct injury to visual cortical networks including the primary visual cortex and optic radiation, often resulting in partial or complete hemianopia. The incidence of significant visual perceptual impairment in victims of TBI exceeds 50-60% in some series. Understanding brain repair processes, and in particular how visual cortical networks reorganize after injury, is an important step in the effort to design treatments aimed at enhancing the ability of the visual system to recover after injury. FMRI can be used to monitor in vivo, non-invasively, global patterns of cortical activity as a function of time following injury, providing a unique opportunity to investigate how repair processes modify cortical networks to promote recovery. Macaque fMRI, in particular, is a valuable animal model because it is close to human behavior and physiology and allows us to standardize the injury we intend to study and apply invasive methods such as electrical stimulation. Here we use macaque fMRI to measure the degree of cortical reorganization observed in higher visual areas following primary visual cortex lesions, and to test whether visual rehabilitation can enhance adaptive reorganization thereby promoting behavioral recovery.
Progress: During the second year of the grant we have accomplished the following:
☐ We have completed the training of Dr Justin Nichols in alert monkey behavioral experimentation and is now able to perform and analyze independently macaque fMRI experiments.
☐ We have trained two animals (Conan and Max) to fixate, and have initiated training of another two animals. Thus we have in training a total of 4 animals, the total number of animals that are required to be trained IN our specific aim #1.
☐ We obtained the first anatomical (T1) and functional (T2*) images from alert monkeys in our setup using</td></tr>
</table>

		the 4-phase array coil that we had constructed during last year. ☐ We have constructed a new larger 4-phase array coil which achieves better brain coverage and which we are currently in the process of testing. ☐ We continued to focus on the development of the appropriate software/hardware environment to allow us to rapidly and efficiently interface the behavior of the primate with the acquisition of fMRI images and/or physiological manipulations (such as electrical stimulation). ☐ On the technical front, we have also completed a new (larger) chair design. Having a larger and more convenient (better coil access) fMRI chair design is essential as the size of the animals is about to reach the limit of the current chair. ☐ We have presented original data at three scientific programs in 2010, including the Howard Hughes Medical Institute, the Human Brain Mapping Conference in Barcelona, Spain, and the Asia-Pacific Conference on Vision, Taipei. **Conclusion**: We have essentially completed setting up the experimental infrastructure necessary for accomplishing specific aim #1 of the SOW, and have already demonstrated that early extrastriate areas can be visually modulated in the chronic absence of area V1 input (see last annual report, Schmid et al., PLoS ONE, 4(5):e5527, 2009). During the next year of the award we expect to continue these experiments in alert and behaving primates, focusing on the effects of behavioral training on cortical reorganization and recovery as outlines in the SOW for accomplishing SA #1. Anticipated technical problems involve the need for a larger primate fMRI chair, which has already been ordered and is under construction.
Neuropsychology Core Provides neuropsychological assessment support to the Integrated Clinical Protocol (Specific Aims 2.1, 2.2, 2.3, and 3.1.2-7)	**Investigators** H. Levin, PhD – PI S. McCauley, PhD E. Wilde, PhD G. Hanten, PhD	**Project Summary** Please refer to Dr. Levin's report for Specific Aim 2.1. Discussion of the Neuropsychology Core is included there.
MRI Imaging Core Assists investigators with their MR imaging and spectroscopy (MRI/MRS) needs, including design and implementation of protocols, acquisition of data, and analysis.	**Investigators** P. Narayana, PhD – PI K. Hasan, PhD K. Bochhorst, PhD R. He, PhD Y. Zhou, PhD	**Project Summary** **Objective**: The overall objective of this core is to assist investigators with their MR imaging/spectroscopy (MRI/S) needs. Specifically, this core will help investigators with the design and implementation of MRI/S protocol, acquisition of the MRI/S data, and analysis. The MRI protocol includes high resolution and three dimensional (3D) image (T2-weighted, susceptibility-weighted, gradient echo, interleaved magnetization transfer), diffusion tensor imaging, and proton magnetic resonance spectroscopy (MRS). The MRI core will provide image data in the format that the individual users prefer and the results of the image analysis. **Specific Aims**: This is a core facility for this project and the specific aims are described in the individual projects. The specific aims of the projects supported by the core are: ***Specific Aim #1.*** To characterize animal models of mild traumatic brain injury (MTBI) in order to mimic the neurobehavioral deficits observed in human MTBI patients (Investigators: Pramod Dash, Ph.D., Douglas DeWitt, Ph.D., Claudia Robertson, M.D). ***Specific Aim #2:*** To improve the diagnosis of MTBI, developing more objective criteria in the early postinjury period as well as in the chronic condition (Investigators: Harvey Levin, Ph.D., Jill Hunter, Ph.D., Elisabeth Wilde, Ph.D.) ***Specific Aim #2.1:*** To study early (<24 hr) diagnosis of MTBI and differentiation of its sequelae related to brain injury from PTSD symptoms present in MTBI and in orthopedic injury (OI) groups studied over a six month follow-up interval. ***Specific aim #2.2:*** To study late (>48hr) diagnosis of MTBI and differentiation of MTBI and PTSD

		(Investigators: Andrew Papanicolaou, Ph.D., Joseph Brier, Ph.D., Eduardo Castillo, Ph.D.) **Progress**: As a part of the overall objective, the MRI core has developed and implemented the scanning protocols and sophisticated analysis techniques. So far 15 subjects (9 mTBI and 6 orthopedic controls) have been scanned. Five of the 9 mTBI patients were scanned twice. The data quality is high and the data transfer protocols are functioning well. While we are up to date with the MRI data, the numbers are too small for a meaningful group analysis. We developed a digital atlas of the normal long Evans rats for group analysis of the DTI and morphometric measures of various structures in an unbiased manner. The analysis of the DTI results clearly demonstrate that hemorrhagic shock exacerbates the injury. We have also completed the necessary parcellation for morphometric analysis. We are currently in the process of analyzing the morphometric data on animals. In summary, all the MRI acquisition and analysis tools are in place. Dr. Narayana, the MRI core Director participates and contributes to the Clinical Working Group and the Model Injury Working Group. At these meetings, various aspects of the projects are discussed and the latest developmental and analysis techniques that are implemented by the MRI core that could help the investigators with various projects are shared. In addition, Dr. Narayana regularly get together with other investigators such as Drs. Levin, Hunter, Wilde, and Robertson to talk about MRI protocols and how they can be improved and data quality issues. Thus the MRI core interacts with the investigators on a continuous and consistent basis. The key research accomplishments of this core include: 1) completed the DTI analysis in four groups of animals, based on group analysis, 2) developed and implemented all necessary image analysis software, 3) implemented processing pipelines for unbiased data analysis, 4) developed specialized animal brain atlas for group analysis, 5) scanned 30 animals (both TBI and sham controls), 6) scanned 12 patients and acquired MRI data, and 7) prepared two manuscripts for publication and presented one abstract at the National Neurotrauma meeting in 2010.
Statistical Core Provides assistance to investigators regarding study design, data management, and statistical analysis, with Integrated Clinical Protocol (Specific Aims 2.1, 2.2, 2.3, and 3.1.2-7) as primary focus; available to all investigators as needed.	**Investigator** P. Swank, PhD -- PI	**Project Summary** **Overview**: As statistician for Specific Aims 2 and 3.1, the Statistical Core has worked mainly with PIs in the Clinical Working Group and data management and planning for the Integrated Clinical Protocol. During the first year of the project we were involved in advising PIs on issues of design and data collection, assistance in setting up data bases and developing programs to aid in data cleaning. During year 2 we have continued advising PIs on these issues and have assisted in setting up the electronic data bases where study data will be stored. For years 3 through 4, we will continue advising on data collection and design issues as well as begin the process of cleaning the data in preparation for data analysis. **Progress**: The Consortium has compiled four clinical projects into the Integrated Clinical Protocol. These projects are: Specific Aims 2.1 (PI Levin), 2.2 (PI Papanicalaou), 2.3 (PI Masel), and 3.1 (PI Robertson). While enrollment has begun and some data has been collected, the electronic data entry system is as yet incomplete so that no data has been entered. We have been working closely with our contracted data management service, Silverwind Research. Progress toward our goal of an electronic data entry system includes: • Creation of paper versions of the CRFs for 26 neuropsychological tests and 11 study visits • Development of electronic CRF versions and database layout, with a fully operational automated system in place by mid-Fall. In addition, we have worked with Dr. Levin's team to establish a mechanism by which reliability of the data collected as part of the clinical protocol and have evaluated several measures for inter-rater reliability. For measures where inter-rater reliability is vulnerable (e.g., the scoring of the Brief Visual-Spatial Memory Test-Revised [BVMT-R] which involves the copying and subsequent drawing of geometric figures from memory), we worked with Dr. McCauley and the Research Coordinators to establish and document a 95% agreement in the subjective scoring of the subject responses on this measure to ensure adequate inter-rater reliability. For Specific Aim 3.1, the clinical trial of atorvastatin in the MTBI subjects, we have prepared a randomization

		scheme and are working closely with the Memorial Hermann Hospital Research Pharmacy and Silverwind Research. We will be monitoring how well the randomization scheme is working and will make any adjustments that are needed in it.
Histopathology Core Will process and subsequently analyze CNS tissues obtained as part of experimental studies, working closely with Investigators from Specific Aim 1 and 3 (those not involved in the Integrated Clinical Protocol)	**Investigator** R. Grill, PhD – PI	**Project Summary** During the second year of funding, the Histopathology Core continued to provide services to those members of the consortium focused on the development and implementation of novel animal models of mild TBI (specific aim 1). Our areas of focus included: 1) Blast-induced injury with Drs. DeWitt of UTMB and Dash of UTH, 2) mild fluid percussion injury with Drs. Dash of UTH and Perez-Polo and Hulsebosch of UTMB, 3) mild cortical contusion injury with Dr. Robertson of Baylor College of Medicine. A summary of results are as follows: Blast: We report multiple blast-induced histological changes occurring over time and detected via immunohistological techniques. These changes include: 1) the generation of an environment enriched in both pro-inflammatory and pro-oxidative conditions, the apparent activation of both glial and immune cell populations and a substantial loss of blood-brain-barrier integrity. Any of these changes, alone, would be of great interest in terms of modeling overall outcome following blast. Together, however, these data suggest that blast-injury elicits profound alterations to the molecular, cellular and structural composition of the brain that may contribute to long-term deficits. Mild Fluid Percussion Injury: We are proceeding with a comparison of mild TBI-induced alterations in rat brain tissue using the blast model (above) as well as the fluid percussion models at UTH and UTMB. We report similar brain response to injury when examined with a batter of immunohistological markers that recognize inflammation, oxidative stress, blood-born as well as local immune response, glial alterations, white matter deficits and alterations in the blood-brain-barrier (BBB). As we are seeing similar responses across the various animal models, we will need to more carefully synchronize our studies to allow a more careful comparison of injury-induced pathological events at later time periods to determine whether mild TBI is capable of eliciting long-term molecular, cellular or tissue-level pathological changes. **Major take-home message:** Despite differences in model of injury, similar responses occur within the brain. The Core wishes to determine the significance, if any, of subtle differences observed between models and also, whether these TBI-induced alterations evolve as a function of time. Cortical contusion injury: The core has continued in its role of processing mild cortical contusion tissue for the Robertson lab, performing lesion volume analysis. As these studies have been blinded, we are unable to report on outcomes. Aim 3.2: We have continued assisting the Dash laboratory in tissue processing for the experiments detailed in Aim 3.2. This work has culminated in a recently published manuscript. Aim 3.8: We have performed tissue processing, immunohistology, and lesion volume measurements for Dr. Kim Tolias of Baylor College of Medicine. Those studies were blinded so we cannot report on outcome.
Neurophysiology Core Will oversee data acquisition and perform analyses of the EEGs obtained as part of the Integrated Clinical Protocol (Specific Aims 2.1, 2.2, 2.3, and 3.1.2-7), including visual analyses and	**Investigators** E. Mizrahi, MD – PI D. Frost, MD D. Friedman, MD R. Hrachovy, M.D J. Slater, MD	**Project Summary** **Introduction**: This report outlines the participation of Eli M. Mizrahi, M.D. and his colleagues in the Mission Connect Mild Traumatic Brain Injury (MTBI) Translational Research Consortium during the time period of August 1, 2009 through July 31, 2010. Dr. Mizrahi is the principal investigator (P.I.) for the Neurophysiology Core which supports the Integrated Clinical Protocol (Specific Aims 2.1, 2.2, 2.3, and 3.1.2-7). The goal of the Neurophysiology Core is to analyze EEG data obtained acutely following injury in subjects and controls. EEG data will be reviewed by both visual and computer analysis. These will be utilized in correlative analysis within the ICP with diffusion tensor imaging (DTI) and cognitive performance measures to achieve the Specific Aims of this project.

computer-based quantitative analyses. These later will include power spectral (Fourier) analysis, period/amplitude analysis and spectral coherence, which will permit EEG activity to be characterized comprehensively in terms of frequency components, amplitude distribution, rhythmicity, continuity, and bilateral symmetry.		**Progress**: Progress for the Core will be described in terms of project organization and personnel, study procedures, quantitative analysis protocols: • Organization and Personnel: The scientific team has been assembled and has begun work with the start of subject enrollment in February 2010 and subsequent availability of clinical electroencephalographic (EEG) data. • Study Procedures: The primary study procedure for the Neurophysiology Core is the recording of the EEG during the Baseline and Three Month visits of the subjects. Thus far, we have: - Established the logistics for recording EEG at the Neurophysiology Laboratories of Memorial Hermann Hospital (MHH), performed pilot/test recordings, and tested our analysis protocols against these. As part of the start-up activities, we identified a number of logistical concerns related to EEG recording procedures. These were addressed as they arose. A working check-list was developed as a guideline for the electroneurodiagnostic technologists. - Developed analysis protocols whereby the EEG is first analyzed visually and scored and then assessed by computer-based quantitative analysis. - Received and analyzed Baseline EEGs for 15 subjects and Three Month EEGs for 4 subjects, as of July 31, 2010 Please see Dr. Levin's report (Specific Aim 2.1) for a full discussion of study procedures and data collection activities. • Quantitative Analysis Protocols: Extensive EEG analysis software/hardware has been developed within the Peter Kellaway Section of Neurophysiology, Department of Neurology, Baylor College of Medicine, using the Matlab programming language, and has been applied to this project. The method of analysis includes The background characteristics of each 30-second EEG sample are determined through the application of a battery of procedures which make use of power spectral (Fourier) analysis, period/amplitude analysis, and spectral coherence.

KEY RESEARCH ACCOMPLISHMENTS:

- Steering Committee: Not applicable
- Model of Injury Working Group: please see investigator reports
- Neuroprotection Working Group: please see investigator reports
- Regeneration Working Group: please see investigator reports
- Clinical Working Group: please see investigator reports

REPORTABLE OUTCOMES:

- Steering Committee: Not applicable
- Model of Injury Working Group: please see investigator reports
- Neuroprotection Working Group: please see investigator reports
- Regeneration Working Group: please see investigator reports
- Clinical Working Group: please see investigator reports

CONCLUSION: In our second year of working together as a Consortium, we have made significant progress, overcoming many of the initial organizational issues inherent in any new consortium. Work is proceeding on all projects, with progress on all projects. Our Working Group structure is providing an effective mechanism for scientific collaboration and coordination of related projects. We have received our initial review from the External Advisory Board and have worked to incorporate their recommendations and feedback into our on-going project activities. We have successfully initiated patient enrollment in our clinical protocol and by adapting to the unique issues at each institution have doubled enrollment over the last 3 months.

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APPENDICES:

Appendix: **Flyer posted in the recruitment sites' Emergency Centers and Pocket Card content**

SUPPORTING DATA: none

Appendix 1:

Flyer posted in the recruitment sites' Emergency Centers

The Mission Connect Mild TBI Research Consortium

We are recruiting patients with Mild TBI and minor Orthopedic Injuries (controls) for a study funded by the Department of Defense, involving cognitive testing, EEG, MRI, and a drug study.

Enrollment Criteria:

- Age 18-50 years
- Injury is < 24 hrs ago
- No other serious injuries
- Will not be admitted
- Fluent in English OR Spanish
- Right-handed (not left-handed)
- Meets either MTBI or Ortho Criteria

•MTBI Criteria

- Has a blunt head injury
- GCS 13-15
- LOC < 30 minutes
- No abnormalities on Head CT

•Ortho Criteria

- No head injury
- Mild extremity injury

To refer a patient, page:

713 318-0138

A Research Nurse will call you back

Melisa Frisby, MSN, RN

Kristen Sheldon, BSN, RN

This study is approved by the IRBs of:

Baylor College of Medicine IRB

H-24162

BCM Baylor College of Medicine

UT Health Science Center IRB

HSC-MS-09-0046

The University of Texas Health Science Center at Houston

Appendix 1 (cont):

Content for Pocket Cards for EC staff

Graphic is sized so that folded document is 3x5 inches, then printed on heavy paper and laminated

The Mission Connect Mild TBI Research Consortium

Please call if you have a patient with:

- Age 18-50 years
- Injury is < 24 hrs ago
- No other serious injuries
- Will not be admitted
- Fluent in English OR Spanish
- Right-handed (not left-handed)
- Meets either MTBI or Ortho Criteria

- **MTBI Criteria**
  - Has a blunt head injury
  - GCS 13-15
  - LOC < 30 minutes
  - No abnormalities on Head CT

- **Ortho Criteria**
  - No head injury
  - Mild extremity injury

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To refer a patient, please page:
713 318-0138

A Research Nurse will call you back
Melisa Frisby, MSN, RN
Kristen Sheldon, BSN, RN

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HSC-MS-09-0046